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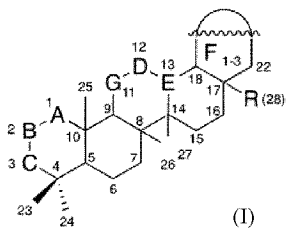
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(54) Title: HYDROXAMATE TRITERPENOID DERIVATIVES



(I)

(57) Abstract: Triterpenoid derivatives and compositions comprising said triterpenoids derivatives of Formula (I) are described, wherein R=-C(O)NHOH. Said triterpenoids and compositions show capacity to bind PHD2, stabilize HIF-1α and HIF-2α proteins, activate the HIF pathway in different cell types, induce angiogenesis in human endothelial vascular cell, show neuroprotective activity in vitro and in vivo, antidiabetic activity and reduce the levels of lipids in vivo, and increase the plasma levels of Erythropoietin in vivo. The triterpenoid derivatives described act also in a selective manner and do not induce Nrf2 activation, NF-κB inhibition, STAT3 inhibition, and TGR5 activation, which are known activities of the natural triterpenoid precursors. Said triterpenoid derivatives are useful in the treatment of conditions and diseases which are responsive to HIF activation such as stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases; and also IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases.

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HYDROXAMATE TRITERPENOID DERIVATIVES

Field of the invention

The present invention relates to novel triterpenoid derivatives, and the synthesis of said compounds. Furthermore, the present invention relates to their use as a medicament and in therapy, particularly as stabilizer of HIF-1α and HIF-2α proteins, activator of the HIF-1 pathway and in treating diseases and conditions responsive to HIF-1α and HIF-2 stabilization. This invention also provides pharmaceutical compositions comprising said compounds and method of treating diseases with said compounds.

Background of the invention

Mammalian cells need to maintain proper oxygen homeostasis in order to execute their aerobic metabolism and energy generation. Since the discovery of the hypoxia-inducible factor (HIF)-1, signaling mechanisms underlying oxygen-sensing by HIF transcription factors have been extensively studied in biological contexts (Wang et al., Redox Rep. 1996 Apr.;2(2) :89-96).HIFs, composed of oxygen-labile α and constitutively expressed β subunits, drive the transcription of numerous genes involved in diverse cellular processes including erythropoiesis, angiogenesis, energy metabolism, ischemia, and inflammation (Semenza et al., J. Biol. Chem. 1994 Sept 23;269(38):237357-63 and Eltzschig et al., Nat. Rev. Drug Discov. 2014 Nov; 13(11):852-69). HIF is present in cells almost exclusively in two forms: HIF-1 and HIF-2. They are heterodimeric transcription factors consisting of a constitutively produced highly abundant HIF-β subunit and either a HIF-1α or HIF-2α partner, in the case of HIF-1 and HIF-2, respectively, sharing 48% sequence homology (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025). HIF-1 is frequently associated with metabolic and vascular responses to hypoxia, whereas HIF-2 is associated with vascular systems but also somewhat more with erythropoiesis (Ratcliffe PJ, J. Clin. Invest. 2007 Apr;117(4):862-5).

The mechanism by which oxygen controls HIF-1α has been revealed by the identification of HIF prolyl hydroxylases (PHDs) (Bruick and McKnight, Science 2001 Nov 9;294(5545):1337-40). Under normoxia, PHD hydroxylates proline residues in the oxygen dependent degradation (ODD) domain of HIF-1α, thereby allowing binding to von Hippel Lindau protein (pVHL)-elonginB-elonginC, leading to active ubiquitination and degradation with a half-life of approximately 5 min. On the other hand, the oxygen deprivation under hypoxia impairs hydroxylation of HIF-1α, by PHDs, resulting in reduced HIF-1α, turnover and subsequent induction of target gene transcription (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025). PHDs belong to

the family of the dioxygenase enzymes that require oxygen, iron, and 2-oxyglutarate (2-OG) for their catalytic activity. Their low affinity to oxygen, which is about 2 to 10 times higher than physiological oxygen concentrations, enables the enzymes to act as oxygen sensors. Three PHD isoforms (PHD1, PHD2, and PHD3) have been identified, and their substrates are known to be quite diverse and isoform-specific (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025 and; Eltzschig et al., Nat. Rev. Drug Discov. 2014 Nov; 13(11):852-69).

PHD2 is considered critical in regulating the HIF pathway. Specifically, enhanced angiogenesis, and increased levels of vascular endothelial growth factor (VEGF)-A and erythropoietin (EPO) were observed in conditional knockout of PHD2 (Takeda et al., Circulation 2007 Aug. 14;116(7):774-81). Such observations, along with the previous report that HIF enhanced EPO release and concomitantly increased erythropoiesis, imply that activation of HIF by modulating PHDs could be beneficial for patients with anemia and ischemia-related diseases. Accordingly, pharmacological approaches to activate the HIF pathway by inhibiting PHD activity have been pursued to treat systemic and local diseases the treatment of which can benefit from HIF activation (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025 and; Eltzschig et al., Nat. Rev. Drug Discov. 2014 Nov; 13(11):852-69).

Hypoxia and inflammation are intimately linked on many levels and have functional roles in many human diseases. Indeed, a wide range of clinical conditions is characterized by hypoxia- or ischemia-driven inflammation or by inflammation-associated hypoxia. Accumulating evidence shows that inflammatory lesions are characterized by the occurrence of tissue hypoxia that is probably a result of increased metabolism and diminished oxygen supply. For example, this is the case during the intestinal inflammation observed in patients suffering from inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease (Karhausen et al., J. Clin Invest. 2004 Oct;114(8):1098-106). Such inflammatory hypoxia is caused by dramatic shifts in metabolic supply and demand ratios. Emerging evidence indicates that concomitant inhibition of PHDs and subsequent stabilization of HIFs during tissue hypoxia could function as an endogenous adaptive response to counterbalance hypoxia-driven inflammation and to restore normal cellular functions (Eltzschig et al., Nat. Rev. Drug Discov. 2014 Nov; 13(11):852-69).

Neuronal damage secondary to brain injuries such as cerebral hypoxia and neurodegenerative process, is a complex process that involves inflammatory changes. The activation of a common mechanism related to survival or cell death, mediated by the stabilization and trans-activation of HIF-1α, has been observed in these conditions. PHDs are the gatekeepers for the oxygen-dependent degradation of HIF-1α and also function as integrated sensors of cellular metabolism

(Aragonés et al., Cell Metab. 2009 Jan 7;9(1):11-22). The phenomenon that hypoxic preconditioning (HP) protects against subsequent severer anoxia was discovered approximately two decades ago. Subsequently, the effects of HP have been studied intensively *in vitro* and *in vivo* models of acute hypoxia. Although the exact mechanisms are not completely disclosed, the underlying molecular mechanisms have been postulated. For example, HP activates a great variety of endogenous protective mediators including HIF-1α and HIF-2α stabilization increasing the capability of cell survival under severe oxygen deprivation (Wu et al., 2012, The protective role of hypoxic preconditioning in CNS, Anoxia, Dr. Pamela Padilla (Ed.), InTech, DOI: 10.5772/27621).

Brain diseases where hypoxia occurs mainly include stroke, cerebral palsy, traumatic injuries etc. Until now, there are no any effective drugs to protect brain from these diseases. Disclosure of the mechanism of HP will contribute to drug discovery for prevention against said diseases. A number of cellular adaptive responses to hypoxia are mediated by HIF-1α and activation of this factor by HP enhances the capability to tolerate severe anoxia or ischemia. The target genes of HIF-1, on the one hand, are involved in energy homeostasis, such as EPO in the regulation of erythropoiesis, vascular endothelial growth factor (VEGF) in angiogenesis, glucose transmitters (GLUTs) in glucose uptake and glycolytic enzymes of anaerobic glycolysis (Speer et al., Free Radio. Biol. Med. 2013 Sep; 62:23-36). Moreover, activation of HIF-1α in oligodendrocytes has been reported to induce EPO, which confers protection in oligodendrocytes against excitotoxicity (Sun et al., J. Neurosci. 2010 Jul 14;30(28):9621-30). In this sense the benefit of EPO in several diseases such as Multiple Sclerosis, stroke and traumatic brain injuries has been also demonstrated (Peng et al., J. Neurosurg. 2014 Sep;121(3):653-64; Ehrenreich et al., Stroke 2009 Dec;40(12): e647-56; Li et al., Ann. Neurol. 2004 Dec;56(6):767-77). In addition, hypoximimetic agents (*i.e.* agents producing the same biological responses as the ones produced when hypoxia occurs) such as desferrioxamine (DFX) protect neuronal insults induced by 3-nitropoionic acid (Yang et al., J. Neurochem. 2005 May;96(3):513-25). Therefore, PHDs inhibition by hypoximimetic small-molecules represents an interesting strategy or the development of neuroprotective therapies for the clinical management of conditions where hypoxia is present such as stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases such as Multiple Sclerosis, Huntington disease, Alzheimer disease and Parkinson disease.

A substantial number of pharmacological studies (generally using nonspecific PDH2 inhibitors) have been conducted in animal models, and a few clinical studies have been performed. Indeed, several companies are involved in the discovery and development of PHD inhibitors for anemia and other indications such as IBD, myocardial ischaemia–reperfusion injury, acute lung injury,

organ transplantation, acute kidney injury and arterial diseases are areas in which PHD inhibitors are actively being pursued by many researchers as a novel therapeutic approach (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025 and; Eltzschig et al., Nat. Rev. Drug Discov. 2014 Nov; 13(11):852-69).

The original description of HIF-selective PHDs as regulators of HIF expression has provided a template for the development of PHD-based molecular tools and therapies. Pharmacological inactivation of the PHDs by 2-OG analogues is sufficient to stabilize HIF-1α, but this action is nonspecific with respect to individual PHD isoforms and *in vitro* studies showed that the ODD sequence of HIF1α is hydroxylated most efficiently by PHD2 (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025). These observations have generated considerable interest in identifying enzyme-modifying small-molecule inhibitors. Indeed, several PHD inhibitor classes have been described, including iron chelators such as DFX, hydralazine, AKB□4924, FG□2229, TM□6008 and l□mimosine; CUL2 deneddylators such as MLN4924; 2-OG mimics such as ximethyloxalylglycine and *N*-oxalyl-d-phenylalanine; PHD active-site blockers such as pyrazolopyridines, 8□hydroxyquinolines, compound A, FG□4497 and TM□6089; and Fe^{2+} substitutes such as Co^{2+}, Ni^{2+} and Cu^{2+}. The mechanism of action of these compounds is based on the observation that the binding of the co-substrate 2-OG to the catalytic domain, which harbours an essential Fe^{2+} ion, is crucial for enzymatic PHD2 activity. Therefore, chemical compounds that structurally mimic 2-OG, such as *N*-oxalylglycine or its precursor DMOG, inhibit PHD2 by blocking the entry of the co-substrate (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025 and; Eltzschig et al., Nat. Rev. Drug Discov. 2014 Nov; 13(11):852-69).

DFX is an hydroxamic acid derivative acting as a metal chelator that is in clinical use for the treatment of acute iron intoxication and of chronic iron overload due to transfusion-dependent anemias. However, DFX is not indicated for the treatment of chronic diseases and it is contraindicated in severe renal diseases since primarily the kidney excretes the drug and the iron chelate. Moreover, DFX only induces HIF-1α stabilization and activation at relatively high concentrations in the cells.

Triterpenoids are widely distributed in edible and medicinal plants and are an integral part of the human diet. Among them, pentacyclic triterpenoids are derived from the linear hydrocarbon squalene and they are highly multifunctional and, thus, have a wide range of commercial applications in the agriculture, food, cosmetics and pharmaceutical sectors as pesticides, drugs, adjuvants, antimicrobials, anticancer agents, surfactants, preservatives, etc. (Sheng et al., Nat. Prod. Rep. 2011 Mar;28(3):543-93; Yadav et al., Toxins (Basel) 2010 Oct;2(10):2428-66). The

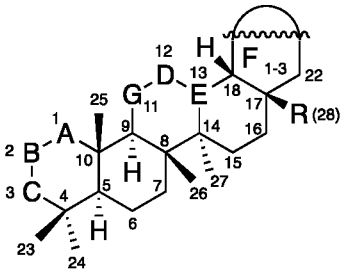
acidic function and hydroxyl (-OH) groups of the triterpenoids cannot interact with the stationary phase, as the two groups are located on the opposite sides of the compound. There are three main triterpene families: oleane, ursane, and lupane triterpenes. The main triterpenoids found in the oleane family are oleanolic acid, maslinic acid, erythrodiol, and β-amyrin; in the ursane family are ursolic acid and uvaol; and in the lupane family are lupeol, betulin, and betulinic acid (Yadav et al., Toxins (Basel) 2010 Oct;2(10):2428-66).

Dietary triterpenoids such as oleanolic, ursolic, betulinic acid and maslinic acids have been extensively studied for bioactivity and pharmaceutical application and they have been widely used also as templates to produce novel derivatives with improved bioactivities. Triterpenoids have been shown to inhibit the activation of the transcription factors NF-κB (Nuclear factor-kappa B) and STAT3 (Signal transducer and activator of transcription 3), to modulate the Nrf2 (NFE2L2 or Nuclear factor (erythroid-derived 2)-like 2) pathway and to bind and activate the bile receptor TGR5 (Sato et al., Biochem. Biophys. Res. Comm. 2007 Nov 3;362(4):793-8). However, the potential activity of triterpenoids on PDH2 inhibition and HIF-1α stabilization has not been described. Surprisingly, ursolic, betulinic, and oleanolic acids, which are the precursors of compounds **II** to **XIV** disclosed herein bind to PHD2 but do not activate the HIF-1α pathway. This finding led to generate hydroxamate derivatives able to bind PHD2, to chelate Fe^{2+} in its active pocket and as a consequence these compounds activate the HIF pathway and stabilize HIF-1α and HIF-2α proteins. Compounds **III**, **IV**, **V**, **VI**, **VIII**, **IX**, **X**, **XI**, and **XIV** and **XV** are novel chemical entities whilst compounds **II**, **VII**, **XII** and **XIII** have been reported to have anticancer activity *in vitro*, which is not related to HIF-1α activation (Wiemann et al., Eur. J. Med. Chem. 2015 Dec 1;106:194-210; Wiemann et al., Bioorg. Med. Chem. Lett. 2016 Feb 1;26(3):907-9; CN102180939 B). In fact, HIF-1α inhibition by some triterpenoids, as opposite to HIF-1α activation, has been suggested as a potential strategy for anti-cancer therapies (Dasgupta et al., Angiogenesis. 2015 Jul; 18(3): 283-99; Jin et al., Arch Pharm Res. 2007 Apr; 30(4): 412-8; Dai et al., J Nat Prod. 2006 Dec; 69(12): 1715-20).

Brief description

Departing from the prior art, the problem of the present invention is to provide novel pentacyclic triterpenoid derivatives (triterpenoid derivatives) that induce HIF-1α and HIF-2α stabilization and activate the HIF pathway.

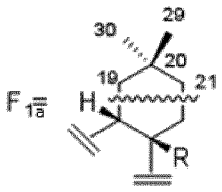
Present invention relates to triterpene derivatives of Formula (**Ia**) or stereoisomers, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof,

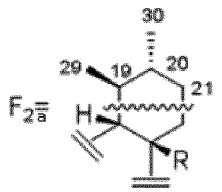


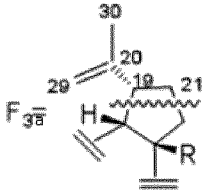
Formula (**Ia**)

wherein independently,

- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;
- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];
- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen; and wherein said heterocyclic ring is a five membered ring comprising one nitrogen and one oxygen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from F_{1a}, F_{2a} or F_{3a};





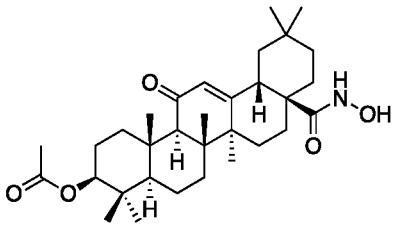


- G is selected from a methylene (-CH_2-) or a carbonyl [-C(=O)-]; and
- R is a hydroxamate group (-CONHOH);

and wherein,

- when C is an acyloxymethine [-CH(OCOR')-], the triterpene derivative of Formula (**Ia**) is

(V)



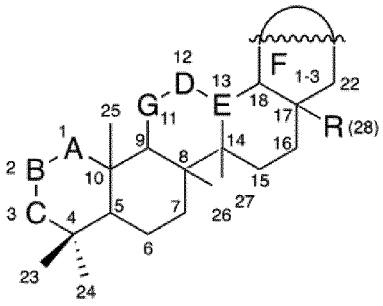
(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide;

- when B is a methylene (-CH_2-), C is a hydroxymethine [-CH(OH)-], D-E is a double carbon-carbon bond, G is a methylene (-CH_2-) and R is a hydroxamate (-CONHOH), F is F_{3a};
- when B is a methylene (-CH_2-), C is a hydroxymethine [-CH(OH)-], D-E is a single carbon-carbon bond, G is a methylene (-CH_2-) and R is a hydroxamate (-CONHOH), F is selected from F_{1a} or F_{2a};
- when B is a methylene (-CH_2-), C is an oxime [-C(=N-OH)-], D-E is a double carbon-carbon bond, G is a methylene (-CH_2-) and R is a hydroxamate (-CONHOH), F is selected from F_{1a} or F_{3a}.

Additionally, present invention refers to pharmaceutical compositions comprising at least one triterpene derivative of Formula (**Ia**), as described above herein, as a first active ingredient and at least one excipient or carrier, and optionally further comprising at least a second active ingredient

Another aspect of present invention refers to the triterpene derivatives of Formula (**Ia**), and the pharmaceutical compositions thereof, as described above herein, for use as a medicament.

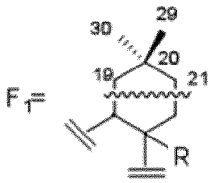
Present invention also refers to triterpene derivatives of Formula (**I**) or stereoisomers, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof,

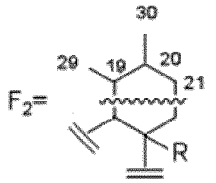


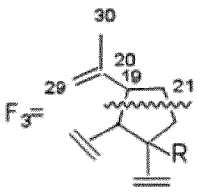
Formula (**I**)

wherein independently,

- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;
- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];
- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen; and wherein said heterocyclic ring is a five-membered ring comprising one nitrogen and one oxygen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from F_1, F_2 or F_3;







- G is selected from a methylene (-CH_2-) or a carbonyl [-C(=O)-]; and
- R is a hydroxamate group (-CONHOH);

for use in the treatment of a condition or disease responsive to the activation of the HIF pathway.

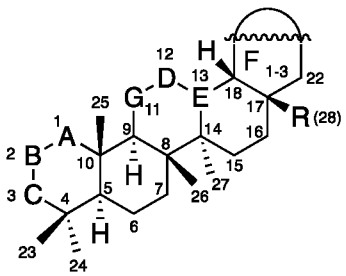
Present invention further relates to pharmaceutical compositions comprising triterpene derivatives, as described above herein, for use in the treatment of conditions or diseases responsive to the activation of the HIF pathway.

Detailed description

Present invention relates to triterpenoid derivatives comprising an hydroxamate functional group at position 28, and compositions comprising said triterpenoids derivatives, which show capacity to bind PHD2, stabilize HIF-1α and HIF-2α proteins, activate the HIF pathway in different cell types, induce angiogenesis in human endothelial vascular cell, show neuroprotective activity *in vitro* and *in vivo*, antidiabetic activity *in vivo*, and increase the plasma levels of Erythropoietin *in vivo*.

Said triterpenoid derivatives act also in a selective manner and do not induce Nrf2 activation, NF-κB inhibition, STAT3 inhibition, and TGR5 activation, which are known activities of their natural triterpenoid precursors. Said triterpenoid derivatives are useful in the treatment of conditions and or diseases responsive to the activation of the HIF pathway. Therefore, present invention relates to diseases, the treatment of which benefits or is responsive to HIF-1α and HIF-2α activation, and thus, present invention does not refer to diseases or conditions the treatment of which benefits or is responsive to HIF-1α inhibition.

More specifically, present invention refers to triterpene derivatives of Formula (**Ia**):

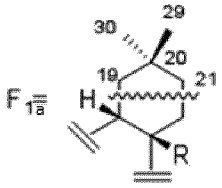


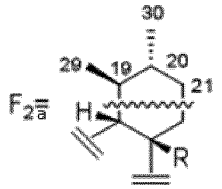
Formula (**Ia**)

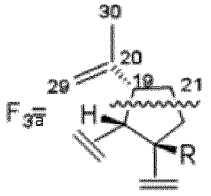
where independently

- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;

- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];
- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from F_{1a}, F_{2a} and F_{3a};







- G is selected from a methylene (-CH_2-) or a carbonyl [-C(=O)-]; and
- R is a hydroxamate group (-CONHOH);

and wherein,

- when B is a methylene (-CH_2-), C is a hydroxymethine [-CH(OH)-], D-E is a double carbon-carbon bond, and R is a hydroxamate (-CONHOH), F is $F_{3a;}$
- when B is a methylene (-CH_2-), C is a hydroxymethine [-CH(OH)-], D-E is a single carbon-carbon bond, and R is a hydroxamate (-CONHOH), F is selected from F_{1a} or F_{2a};
- when B is a methylene (-CH_2-), C is an oxime [-C(=N-OH)-], D-E is a double carbon-carbon bond, and R is a hydroxamate (-CONHOH), F is selected from F_{2a} or F_{3a}.

In one embodiment the triterpene derivatives of Formula (**Ia**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring.

In one embodiment of the triterpene derivatives of Formula (**Ia**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring comprising two nitrogen atoms.

In one embodiment of the triterpene derivatives of Formula (**Ia**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring comprising one nitrogen and one oxygen.

The triterpene derivatives of Formula (**Ia**), as disclosed above herein, also comprise their analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates.

F

or the purposes of present description, the term “analogue/s” refers to any entity structurally derived or homologous to the compounds disclosed in present invention.

In the context of present description, the term “derivative/s” of the compounds disclosed herein should be interpreted as any triterpenoid analogue, always substituted in the position 28, according to the definition of R above herein, which provides with the pharmacological properties described herein to the triterpenoid compounds, but also featuring other substitutions in the other positions of the triterpenoid molecule, different to the substitutions described herein.

The term “tautomers” are constitutional isomers of organic compounds that readily interconvert by a chemical process (tautomerization).

The term "isomer" is defined formally by the IUPAC (International Union of Pure and Applied Chemistry) as one of several species (or molecular entities) that have the same atomic composition (molecular formula) but different formulae or different stereochemical formulae, whereas "stereoisomers" is defined formally by the IUPAC as compounds, which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

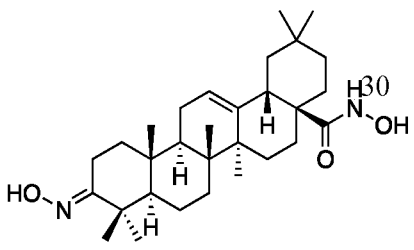
As used herein "polymorph" refers to crystalline forms having the same chemical composition but different spatial arrangements of the molecules, atoms, and/or ions forming the crystal.

The term "pharmaceutically acceptable salt" refers to any pharmaceutically acceptable salt, which upon administration to the patient is capable of providing (directly or indirectly) a compound as described herein. Such salts preferably are acid addition salts with physiologically acceptable organic or inorganic acids. Examples of the acid addition salts include mineral acid addition salts such as, for example, hydrochloride, hydrobromide, hydroiodide, sulphate, nitrate, phosphate, and organic acid addition salts such as, for example, acetate, trifluoroacetate, maleate, fumarate, citrate, oxalate, succinate, tartrate, malate, mandelate, methanesulphonate and p-toluenesulphonate. Examples of the alkali addition salts include inorganic salts such as, for example, sodium, potassium, calcium and ammonium salts, and organic alkali salts such as, for example, ethylenediamine, ethanolamine, N,N-dialkylenethanolamine, triethanolamine and basic aminoacids salts. However, it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the invention since those may be useful in the preparation of pharmaceutically acceptable salts. Procedures for salt formation are conventional in the art.

The term "solvate" in accordance with this invention should be understood as meaning any form of the active compound in accordance with the invention in which said compound is bonded by a non-covalent bond to another molecule (normally a polar solvent), including especially hydrates and alcoholates.

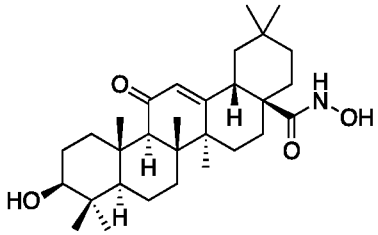
In a preferred embodiment, the triterpene derivatives disclosed refer to the compounds of Formula (**III**), (**IV**), (**V**), (**VI**), (**VIII**), (**IX**), (**X**), (**XI**), (**XIV**), (**XV**), or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof.

(III)



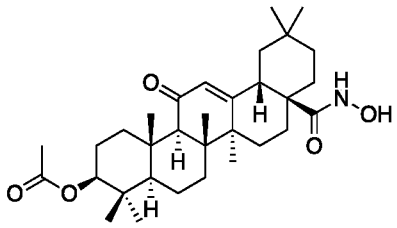
3-Hydroxyimino-*N*-hydroxy-olean-12-en-28-amide

(IV)



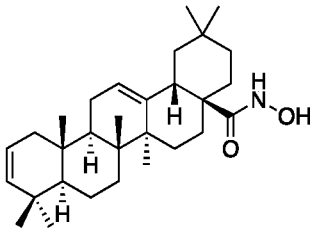
(3β) 3-Hydroxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(V)



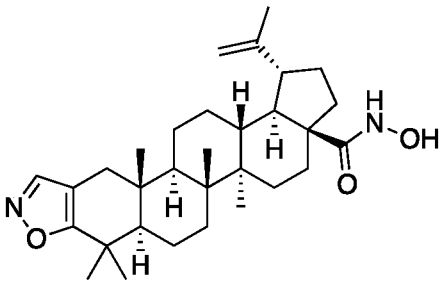
(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(VI)



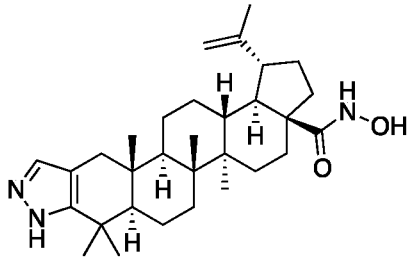
Oleana-2,12-dien-*N*-hydroxy-28-amide

(VIII)



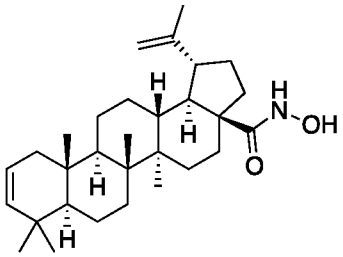
Lup-2-eno[2,3-d]isoxazol-*N*-hydroxy-28-amide

(IX)



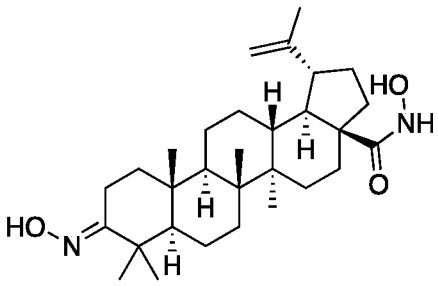
1'H-Lup-20(29)-eno[3,2-c]pyrazol-*N*-hydroxy-28-amide

(X)



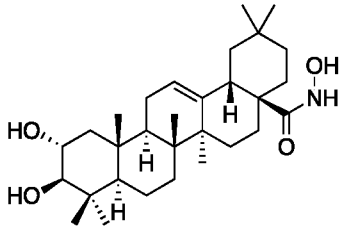
Lupa-2,20(29)-dien-*N*-hydroxy-28-amide

(XI)



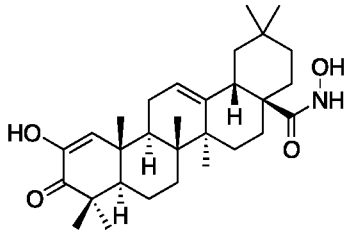
3-Oxo-lup-20(29)-en-28-acid

(XIV)



(2α,3β) 2,3-Dihydroxy-*N*-hydroxy-olean-12-en-28-amide

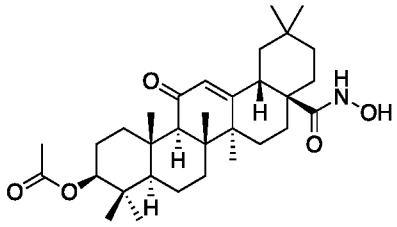
(XV)



2-Hydroxy-3-oxo-oleana-1,12-dien-*N*-hydroxy-28-amide

In a preferred embodiment of the triterpene derivatives of Formula (**Ia**), when C is an acyloxymethine [-CH(OCOR')-], the triterpene derivative of Formula (**Ia**) is

(V)



(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide;

As shown in the examples and figures of present description, the modifications comprised in the general Formula (**Ia**) confer the compounds disclosed herein with the capacity to bind PHD2, stabilize HIF-1α and HIF-2α proteins, activate the HIF pathway in different cell types, induce angiogenesis in human endothelial vascular cell, show neuroprotective activity *in vitro* and *in vivo*, antidiabetic activity *in vivo*, and increase the plasma levels of Erythropoietin *in vivo*. Said compounds of Formula (**Ia**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, act in a selective manner and do not induce Nrf2 activation, NF-κB inhibition, STAT3 inhibition, and TGR5 activation, which are known activities of their natural triterpenoid precursors.

An embodiment disclosed herein refers to compositions, particularly pharmaceutical compositions, comprising at least a compound of Formula (**Ia**) or analog, derivative, tautomeric form, isomer, stereoisomer, polymorph, pharmaceutically acceptable salt or pharmaceutically acceptable solvate thereof, as described above herein, and at least one excipient or carrier.

Said excipient or carrier refers, for the purpose of present invention, to an inert ingredient such as, but not limited to, cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Any pharmacologically acceptable buffer may be used, such as TRIS or any phosphate buffer.

Another embodiment disclosed herein refers to compositions, particularly pharmaceutical compositions, comprising at least a compound of Formula (**Ia**) or analog, derivative, tautomeric form, isomer, stereoisomer, polymorph, pharmaceutically acceptable salt or pharmaceutically acceptable solvate thereof, as described above herein, and at least a second active compound having additive or synergistic biological activities.

For the purposes of present description, the term "active compound or active principle" should be taken as synonyms and mean a chemical entity which exerts therapeutic effects when administered to human or animal beings.

A further embodiment disclosed herein refers to the compounds of Formula (**Ia**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, of present invention, for use as a medicament.

A further embodiment disclosed herein refers to the pharmaceutical compositions described above herein, comprising the compounds of Formula (**Ia**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, of present invention, for use as a medicament.

Said compounds of Formula (**Ia**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, show capacity to bind PHD2, stabilize HIF-1α and HIF-2α proteins, activate the HIF pathway in different cell types, induce angiogenesis in human endothelial vascular cell, show neuroprotective activity *in vitro* and *in vivo*, antidiabetic activity *in vivo*, and increase the plasma levels of Erythropoietin *in vivo*. Said compounds of Formula (**Ia**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, act in a selective manner and do not induce Nrf2 activation, NF-κB inhibition, STAT3 inhibition, and TGR5 activation, which are known activities of their natural triterpenoid precursors.

Said triterpenoid derivatives of Formula Ia, and pharmaceutical compositions thereof, are useful in the treatment of conditions and diseases which are responsive to the activation of the HIF pathway.

Therefore, the triterpene derivatives of Formula (**Ia**) are useful in conditions or diseases responsive to the activation of the HIF pathway, wherein thus, the treatment of said conditions or diseases benefits or is responsive to the activation of the HIF pathway.

According to present disclosure, the conditions and diseases responsive to the activation of the HIF pathway do not include solid tumors. This is the case since solid tumors develop neoangiogenesis that facilitate metastatic spread and therefore HIF-1 inhibitors are being developed as anticancer drugs to block abnormal vessel growth and function (Potente et al. Cell. 2011 Sep 16;146(6):873-87).

Particularly, one embodiment discloses the triterpene derivatives of Formula (**Ia**) described above herein, and pharmaceutical compositions thereof, for use in the treatment of a range of conditions or diseases which are responsive to the activation of the HIF pathway which comprise but are not limited to stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases. Examples of neurodegenerative diseases include but are not limited to Multiple Sclerosis, Huntington disease, Alzheimer disease or Parkinson disease.

For the purposes of present description stroke refers to neurological deficit of cerebrovascular cause. Strokes can be classified into two major categories: ischemic and hemorrhagic. Ischemic strokes are caused by interruption of the blood supply to the brain, while hemorrhagic strokes result from the rupture of a blood vessel or an abnormal vascular structure.

For the purposes of present description cerebral palsy refers to a group of permanent movement disorders that appear in early childhood and are caused by abnormal development or damage to the parts of the brain that control movement, balance, and posture.

For the purposes of present description traumatic injuries refer to physical injuries of sudden onset and severity, which require immediate medical attention. Traumatic injuries are the result of a wide variety of blunt, penetrating and burn mechanisms. Said traumatic injuries include motor vehicle collisions, sports injuries, falls, natural disasters and a multitude of other physical injuries.

Another embodiment discloses the triterpene derivatives of Formula (**Ia**) described above herein, and pharmaceutical compositions thereof, for use in the treatment of a range of conditions or diseases which are responsive to the activation of the HIF pathway, wherein said conditions or diseases comprise, but are not limited to IBD, myocardial ischaemia–reperfusion injury, acute

lung injury, , diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases. More preferably said condition or disease is selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, organ transplantation, acute kidney injury or arterial diseases.

For the purposes of present description IBD refers to a group of inflammatory conditions of the colon and small intestine. Crohn's disease and ulcerative colitis are the principal types of inflammatory bowel disease within others.

For the purposes of present description myocardial ischaemia–reperfusion injury refers to the tissue damage caused when blood supply returns to the tissue after a period of myocardial ischemia or lack of oxygen (anoxia, hypoxia).

For the purposes of present description acute lung injury refers to a condition that is characterized by acute severe hypoxia and where its diagnosis is based on the presence of non-cardiogenic pulmonary oedema and respiratory failure in a critically ill patient.

For the purposes of present description infectious diseases refer to diseases caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi, and wherein said diseases can be spread, directly or indirectly, from one person to another.

For the purposes of present description diabetic and chronic wounds refer to wounds, which may be caused by a diabetic condition, that do not heal in an orderly set of stages and in a predictable amount of time the way most wounds do. In general, wounds that do not heal within three months are often considered chronic

For the purposes of present description acute kidney injury refers an abrupt loss of kidney function from numerous causes that develops within 7 days. Said abrupt loss of kidney function causes damage to the kidney tissue and it is generally caused by decreased renal blood flow (renal ischemia).

For the purposes of present description arterial diseases include a class of diseases that involve the blood vessels.

Particularly, one embodiment discloses the compounds of Formula (**Ia**) as described herein, and pharmaceutical compositions thereof, for use in the treatment of a condition or disease responsive to the activation of the HIF pathway selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases. In a preferred embodiment said condition or disease responsive to the activation of the HIF pathway selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, organ transplantation, acute kidney injury or arterial diseases

One embodiment of present invention, also discloses the compounds of Formula (Ia), as described above herein, and pharmaceutical compositions thereof, for use in the treatment of a condition or disease responsive to the activation of the HIF pathway wherein said condition or disease responsive to the activation of the HIF pathway a metabolic pathology. In a preferred embodiment said metabolic pathology is diabetes. In another preferred embodiment said metabolic pathology is a lipid metabolism disorder. In yet another preferred embodiment said metabolic pathology is hyperlipidemia. In another preferred embodiment said metabolic pathology is hypertriglyceridemia. It has been described that PHD2 inhibitors improve glucose and lipid metabolism and protects against obesity and metabolic syndrome (Rahtu-Korpela et al. Diabetes. 2014 Oct;63(10):3324-33).

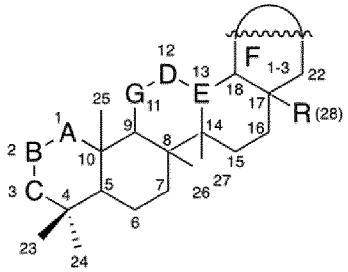
The term "metabolic pathologies" refers to pathologies where the normal metabolic process has been altered.

The term "diabetes", for the purposes of present invention, refers a group of metabolic disorders in which high blood sugar levels are present over a prolonged period. Diabetes is due to either a low production of insulin by the pancreas or to an inappropriate response of the body to the insulin produced.

"Hyperlipidemia" refers to a condition wherein the subject presents an abnormally elevated level of any, or all, lipids, or lipoproteins in the blood.

"Hypertriglyceridemia" refers to a condition wherein the subject presents high blood levels of triglycerides. Elevated levels of triglycerides are associated with atherosclerosis, even in the absence of hypercholesterolemia (high cholesterol levels), and predispose to cardiovascular disease. Very high triglyceride levels also increase the risk of acute pancreatitis.

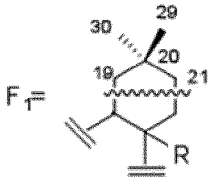
Another embodiment disclosed herein refers to triterpene derivatives of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof,

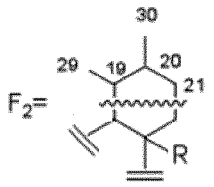


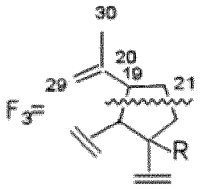
Formula (**I**)

wherein independently,

- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;
- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];
- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from F_1, F_2 or F_3;







- G is selected from a methylene (-CH_2-) or a carbonyl [-C(=O)-]; and
- R is a hydroxamate group (-CONHOH);

for use in the treatment of conditions or diseases responsive to the activation of the HIF pathway.

Therefore, the triterpene derivatives of Formula (I) are useful in conditions or diseases responsive to the activation of the HIF pathway, wherein thus, the treatment of said conditions or diseases benefits or is responsive to the activation of the HIF pathway.

The triterpene derivatives of Formula (**I**), as disclosed above herein, also comprise their analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable

salts or pharmaceutically acceptable solvates, as defined above herein for the purposes of present invention. In particular, the triterpene derivatives of Formula (I) of present invention, as disclosed above herein, comprise all stereoisomers, and any pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof.

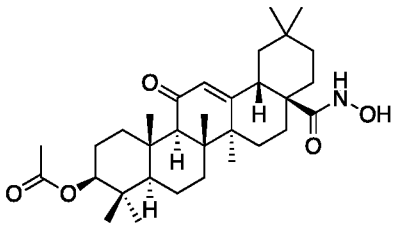
In one embodiment the triterpene derivatives of Formula (**I**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring.

In one embodiment of the triterpene derivatives of Formula (**I**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring comprising two nitrogen atoms.

In one embodiment of the triterpene derivatives of Formula (**I**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring comprising one nitrogen and one oxygen.

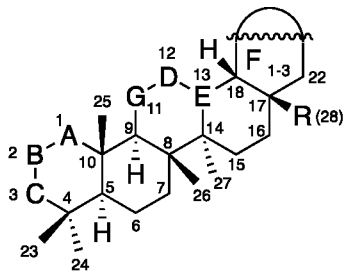
In another embodiment of the triterpene derivatives of Formula (**I**), when C is an acyloxymethine [-CH(OCOR')-], the triterpene derivative of Formula (**I**) is

(V)



(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide;

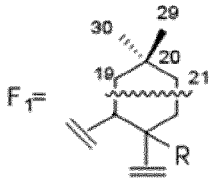
One embodiment of the triterpene derivatives of Formula (I) refers to the stereoisomer thereof Formula (**Ia**):

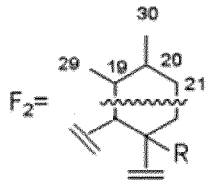


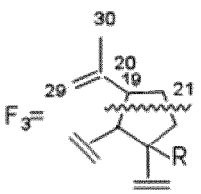
Formula (**Ia**)

where independently

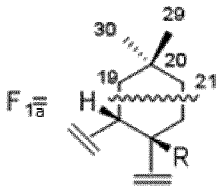
- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;
- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];
- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from

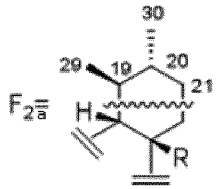


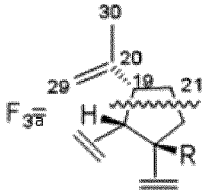




and wherein F_1 is F_{1a}, F_2 is F_{2a} and F_3 is F_{3a};



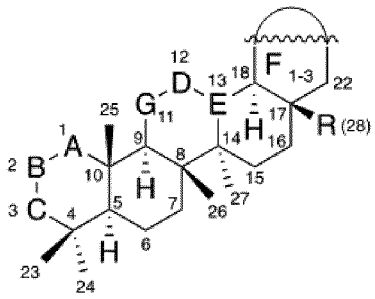




- G is selected from a methylene (-CH_2-) or a carbonyl [-C(=O)-]; and
- R is a hydroxamate group (-CONHOH);

for use in the treatment of conditions or diseases responsive to the activation of the HIF pathway.

Another embodiment of the triterpene derivatives of Formula (I) refers to the stereoisomer thereof Formula (Ib):

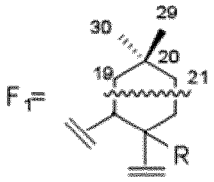


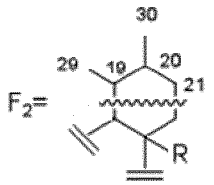
Formula (Ib)

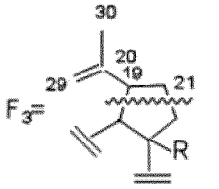
wherein independently,

- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;
- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];

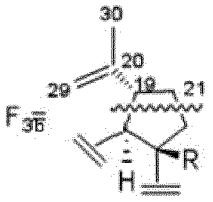
- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from F_1, F_2 or F_3;







and wherein when F is F_3, F_3 is F_{3b}:



for use in the treatment of a condition or disease responsive to the activation of the HIF pathway.

The compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, described in present invention, show capacity to:

- bind PHD2, as shown in Example 2 disclosed below herein,

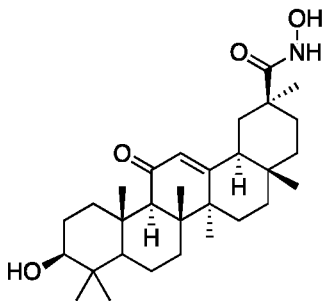
- stabilize HIF-1α and HIF-2α proteins, as shown in Example 4 disclosed below herein,
- activate the HIF pathway in different cell types, as shown in Example 3 disclosed below herein,
- induce angiogenesis in human endothelial vascular cell, as shown in Example 5 disclosed below herein,
- show neuroprotective activity *in vitro* and *in vivo*, as shown in Example 7 disclosed below herein, and
- increase the plasma levels of Erythropoietin *in vivo*, as shown in Example 6 disclosed below herein;
- show antidiabetic activity and reduce the levels of lipids *in vivo*, as shown in Example 9 disclosed below herein.

Said triterpenoid derivatives act also in a selective manner and do not induce Nrf2 activation, NF-κB inhibition, STAT3 inhibition, and TGR5 activation, which are known activities of their natural triterpenoid precursors (as shown in Example 3 of present description).

Example 3 of present invention shows, as mentioned above, how the compounds of Formula (I) activate the HIF pathway in different cell types. Example 3 also tested two comparative compounds XX and CDDO-ME.

Comparative compound XX, derived from glycyrrethinic acid, comprises an hydroxamate group in position 20 and thus not in position 28 as the compounds of present invention.

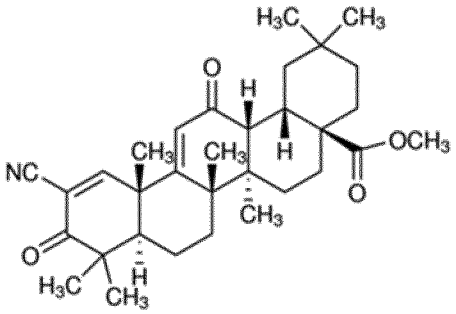
(XX)



(3β) 3-Hydroxy-11-oxo-olean-12-en-*N*-hydroxy-29-amide

The results of Example 3 indicate that the change of the position of the hydroxamate, from the position 28 featured by the compounds of present invention, results in the absence of the activation of the HIF pathway.

On the other hand, comparative compound CDDO-Me (2-Cyano-3,12-dioxo-oleana-1,9(11)-dien-28-oic acid methyl ester, CAS num. 218600-53-4), is an oleanolic acid derivative, but which includes a cyano group in position 2 (substituent at the position B of Formula I) which is a substituent substantively different from the ones featured in said position by the compounds of Formula I disclosed in present invention.



CDDO-Me

The results of Example 3 show that CDDO-Me does not provide activation of the HIF pathway and thus, none of comparative compounds XX and CDDO-Me, which do not present the structural characteristics of the compounds of Formula I, provide activation of the HIF pathway.

Therefore, the triterpenoid derivatives defined by Formula I, disclosed in present invention, are useful in the treatment of conditions or diseases which are responsive to the activation of the HIF pathway, i.e., conditions or diseases the treatment of which benefits from HIF-1α and HIF-2α activation.

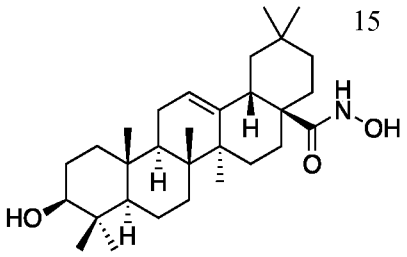
Particularly, one embodiment discloses the triterpene derivatives of Formula (**I**) described above herein for use in the treatment of conditions or diseases which are responsive to the activation of the HIF pathway, which comprise but are not limited to stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases. Examples of neurodegenerative diseases include but are not limited to Multiple Sclerosis, Huntington disease, Alzheimer disease or Parkinson disease.

Another embodiment discloses the triterpene derivatives of Formula (**I**) described above herein, for use in the treatment of conditions or diseases which are responsive to the activation of the HIF pathway, which comprise but are not limited to IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases. More preferably said condition or disease is selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, organ transplantation, acute kidney injury or arterial diseases.

One embodiment of present invention, also discloses the compounds of Formula (I), as described above herein, for use in the treatment of a condition or disease responsive to the activation of the HIF pathway wherein said condition or disease responsive to the activation of the HIF pathway a metabolic pathology. In a preferred embodiment said metabolic pathology is diabetes. In another preferred embodiment said metabolic pathology is a lipid metabolism disorder. In yet another preferred embodiment said metabolic pathology is hyperlipidemia. In another preferred embodiment said metabolic pathology is hypertriglyceridemia.

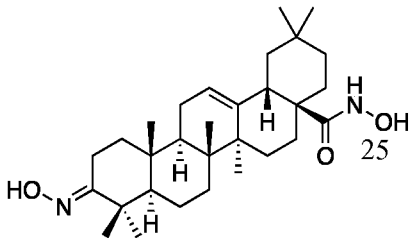
Another embodiment disclosed herein refers to the compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof selected from (**II**), (**III**), (**IV**), (**V**), (**VI**), (**VII**), (**VIII**), (**IX**), (**X**), (**XI**), (**XII**), (**XIII**), (**XIV**) or (**XV**).

(II)



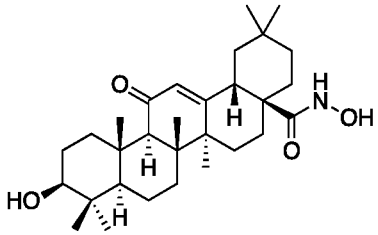
(3β) 3-Hydroxy-*N*-hydroxy-olean-12-en-28-amide

(III)



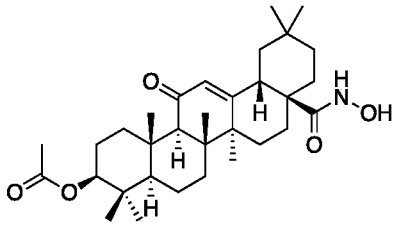
3-Hydroxyimino-*N*-hydroxy-olean-12-en-28-amide

(IV)



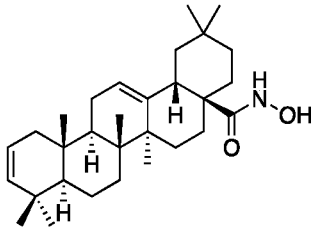
(3β) 3-Hydroxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(V)



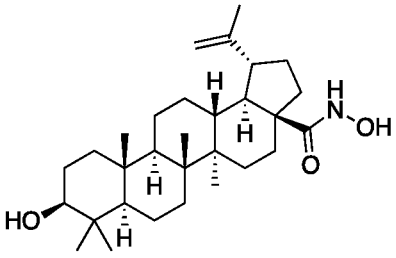
(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(VI)



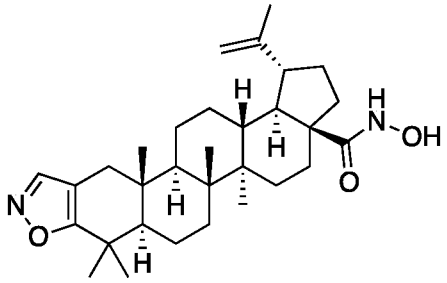
Oleana-2,12-dien-*N*-hydroxy-28-amide

(VII)



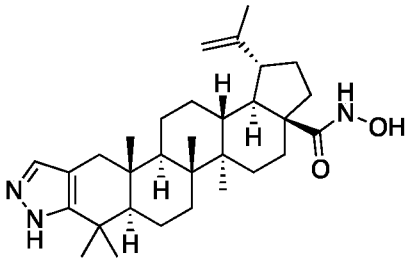
(3β) 3-Hydroxy-*N*-hydroxy-lup-20(29)-en-28-amide

(VIII)



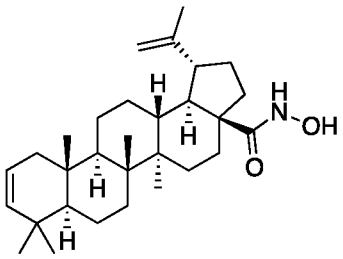
Lup-2-eno[2,3-d]isoxazol-*N*-hydroxy-28-amide

(IX)



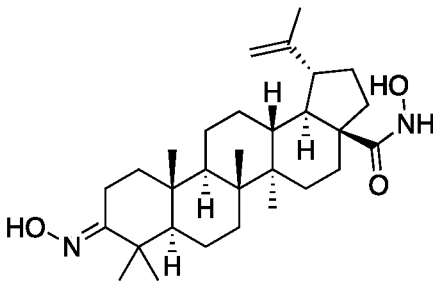
1'H-Lup-20(29)-eno[3,2-c]pyrazol-*N*-hydroxy-28-amide

(X)



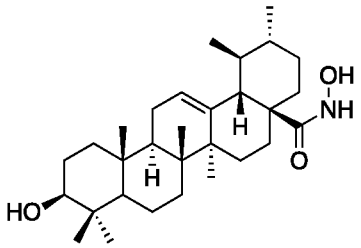
Lupa-2,20(29)-dien-*N*-hydroxy-28-amide

(XI)



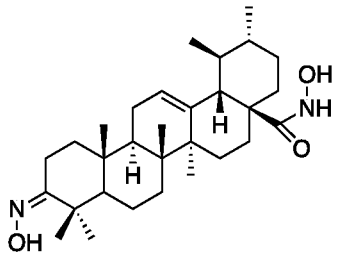
3-Hydroxyimino-*N*-hydroxy-lup-20(29)-en-28-amide

(XII)



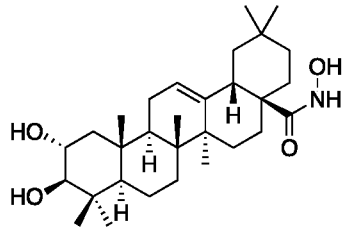
(3β) 3-Hydroxy-*N*-hydroxy-urs-12-en-28-amide

(XIII)



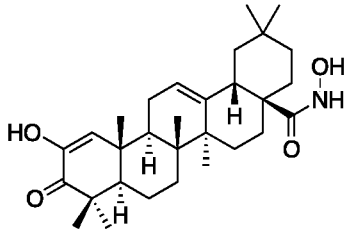
3 Hydroxyimino-*N*-hydroxy-urs-12-en-28-amide

(XIV)



(2α,3β) 2,3-Dihydroxy-*N*-hydroxy-olean-12-en-28-amide

(XV)



2-Hydroxy-3-oxo-oleana-1,12-dien-*N*-hydroxy-28-amide

for use in the treatment of conditions or diseases responsive to the activation of the HIF pathway. A preferred embodiment refers to the compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof selected from (**II**), (**III**), (**IV**), (**V**), (**VI**), (**VII**), (**VIII**), (**X**), (**XI**), (**XII**), (**XIII**), (**XIV**) or (**XV**) for use in the treatment of conditions or diseases responsive to the activation of the HIF pathway.

The above mentioned compounds **II** to **XV**, or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, show capacity to bind PHD2, stabilize HIF-1α and HIF-2α proteins, activate the HIF pathway in different cell types, induce angiogenesis in human endothelial vascular cell, show neuroprotective activity *in vitro* and *in vivo*, antidiabetic activity and reduce levels of lipids *in vivo*, and increase the plasma levels of Erythropoietin *in vivo*. Said compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, act in a selective manner and do not induce Nrf2 activation, NF-κB inhibition, STAT3 inhibition, and TGR5 activation, which are known activities of their natural triterpenoid precursors.

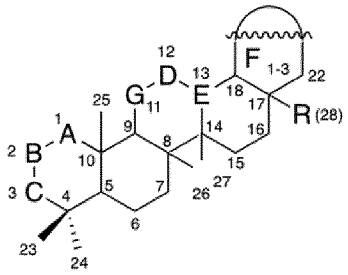
As shown in Example 1, starting from a known derivative of a natural triterpenic acid (betulinic, oleanolic, ursolic, or maslinic acids) or a natural triterpenic acid itself, and ending with the compounds of general Formula (**I**), a common synthetic route was used to convert the acid group of position 28 into the hydroxamate derivative, wherein, depending on the functionalization of the final compound of Formula (**I**) other extra synthetic steps were carried out for each of the specific compounds of Formula (**I**).

Compounds **III** to **VI** of present invention can be synthesized starting from known derivatives of natural oleanolic acid. Compounds **VIII** to **XI** of the present invention can be synthesized by starting from natural betulinic acid or a known derivative thereof. Compounds **XIV** and **XV** of the present invention can be synthesized starting from a known derivative of natural maslinic acid.

Example 1 also describes compounds **XVII** and **XVIII,** and **XIX** that are novel compounds intermediate in the synthesis of compounds **XV** and **XIV** respectively.

Another embodiment disclosed herein refers to the pharmaceutical compositions comprising at least one triterpene derivative of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers,

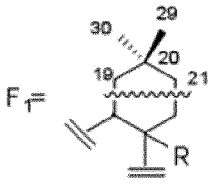
stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof,

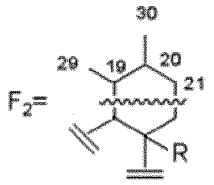


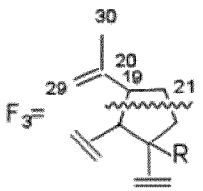
Formula (**I**)

wherein independently,

- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;
- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];
- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from F_1, F_2 or F_3;







- G is selected from a methylene (-CH_2-) or a carbonyl [-C(=O)-]; and
- R is a hydroxamate group (-CONHOH);

for use in the treatment of conditions or diseases responsive to the activation of the HIF pathway.

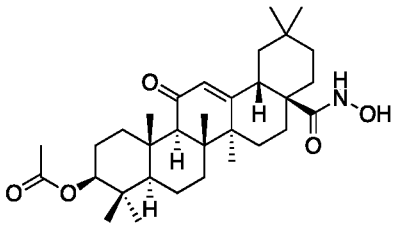
In one embodiment of the pharmaceutical compositions comprising the triterpene derivatives of Formula (**I**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring.

In one embodiment of the pharmaceutical compositions comprising the triterpene derivatives of Formula (**I**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring comprising two nitrogen atoms.

In one embodiment of the pharmaceutical compositions comprising the triterpene derivatives of Formula (**I**), as disclosed above herein, B-C can be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen, and wherein said heterocyclic ring is a five-membered ring comprising one nitrogen and one oxygen.

In one embodiment of the triterpene derivatives of Formula (**I**), when C is an acyloxymethine [-CH(OCOR')-], the triterpene derivative of Formula (**I**) is

(V)



(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide;

Said pharmaceutical compositions comprising compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, show capacity to bind PHD2, stabilize HIF-1α and HIF-2α proteins, activate the HIF pathway in different cell types, induce angiogenesis in human endothelial vascular cell, show neuroprotective activity *in vitro* and *in vivo*, antidiabetic activity

and reduce the levels of lipids *in vivo*, and increase the plasma levels of Erythropoietin *in vivo*. Said compositions comprising compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, act in a selective manner and do not induce Nrf2 activation, NF-κB inhibition, STAT3 inhibition, and TGR5 activation, which are known activities of their natural triterpenoid precursors.

Another embodiment disclosed herein refers to the pharmaceutical compositions comprising at least one triterpene derivative of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, as described above herein, for use in the treatment of conditions or diseases responsive to the activation of the HIF pathway, which comprise but are not limited to stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases; or for use in the treatment of conditions or diseases responsive to the activation of the HIF pathway, which comprise but are not limited to IBD, myocardial ischaemia–reperfusion injury, acute lung injury, , diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases. More preferably said condition or disease is selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, organ transplantation, acute kidney injury or arterial diseases.

Another embodiment disclosed herein refers to the pharmaceutical compositions comprising at least one triterpene derivative of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, as described above herein, for use in the treatment of a condition or disease responsive to the activation of the HIF pathway wherein said condition or disease responsive to the activation of the HIF pathway a metabolic pathology. In a preferred embodiment said metabolic pathology is diabetes. In another preferred embodiment said metabolic pathology is a lipid metabolism disorder. In yet another preferred embodiment said metabolic pathology is hyperlipidemia. In another preferred embodiment said metabolic pathology is hypertriglyceridemia.

Said compositions may further comprise another active ingredient which exerts therapeutic effects when administered to human or animal beings.

Typical compositions include the compounds of Formula (**I**), or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, described above herein associated with pharmaceutically acceptable excipients, which may be a carrier or a diluent, as a way of example. Such compositions can be in the form of a capsule, sachet, paper or other container. In making the compositions, conventional techniques for the preparation of pharmaceutical compositions may be used. For example, the compounds of Formula (**I**) disclosed above herein may be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier that may be in the form of an ampoule, capsule, sachet, paper, or other container. When the carrier serves as a diluent, it may be solid, semi-solid, or liquid material that acts as a vehicle, excipient, or medium for the active compound. The compounds of Formula (**I**) as described above herein can be adsorbed on a granular solid container for example in a sachet. Some examples of suitable carriers are water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, peanut oil, olive oil, lactose, terra alba, sucrose, cyclodextrin, amylose, magnesium stearate, talc, gelatin, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, polyoxyethylene, hydroxymethylcellulose, and polyvinylpyrrolidone. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax. Said compositions may also include wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavouring agents. The compositions of the invention may be formulated so as to provide quick, sustained, or delayed release of the compounds of Formula (**I**) disclosed herein after administration to the patient by employing procedures well known in the art.

The pharmaceutical compositions can be sterilized and mixed, if desired, with auxiliary agents, emulsifiers, salt for influencing osmotic pressure, buffers and/or colouring substances and the like, which do not deleteriously react with the compounds disclosed above herein.

One preferred embodiment disclosed herein refers to the route of administration, that may be any route which effectively transports the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein, to the appropriate or desired site of action, such as oral, nasal, topical, pulmonary, transdermal or parenteral, e. g., rectal, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic solution or an ointment.

For nasal administration, the compositions may contain the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein, dissolved or suspended in a liquid carrier, in particular an aqueous carrier, for aerosol application. The carrier may contain additives such as solubilizing agents, e.g., propylene glycol, surfactants, absorption enhancers such as lecithin (phosphatidylcholine), or cyclodextrin, or preservatives such as parabens.

To prepare topical compositions, the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein, is placed in a dermatological vehicle as is known in the art. The amount of the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein to be administered and the compound's concentration in the topical formulations depend upon the vehicle, delivery system or device selected, the clinical condition of the patient, the side effects and the stability of the compound in the formulation. Thus, the physician employs the appropriate preparation containing the appropriate concentration of the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein, and selects the amount of formulation administered, depending upon clinical experience with the patient in question or with similar patients.

For ophthalmic applications, the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein, is formulated into solutions, suspensions, and ointments appropriate for use in the eye. The concentrations are usually as discussed above herein for local preparations.

For oral administration, either solid or fluid unit dosage forms can be prepared. For preparing solid compositions such as tablets, the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein, is mixed into formulations with conventional ingredients such as talc, magnesium stearate, di-calcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, acacia, methylcellulose, and functionally similar materials as pharmaceutical diluents or carriers.

Capsules are prepared by mixing the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein with an inert pharmaceutical diluent, and filling the mixture into a hard gelatin capsule of appropriate size. Soft gelatin capsules are prepared by machine encapsulation of slurry of the compound of Formula (**I**) with an acceptable vegetable oil, light liquid petrolatum or other inert oil. Fluid unit dosage forms for oral administration such as syrups, elixirs and suspensions can be prepared. The water-soluble forms can be dissolved in an aqueous vehicle together with sugar, aromatic flavoring agents and preservatives to form syrup. An elixir is prepared by using a hydroalcoholic (e. g., ethanol) vehicle with suitable sweeteners such as sugar and saccharin, together with an aromatic flavoring agent. Suspensions can be prepared with an aqueous vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

Appropriate compositions for parenteral use are apparent to the practitioner of ordinary skill, such as the use of suitable injectable solutions or suspensions. The composition, which is sterile, is suitable for various topical or parenteral routes including intra-dermal, intramuscular, intravascular, and subcutaneous.

In addition to the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein, the compositions may include, depending on the composition and mode of delivery desired, pharmaceutically- acceptable, non-toxic carriers or diluents, which include vehicles commonly used to form pharmaceutical compositions for animal or human administration. The diluents are selected so as not to unduly affect the biological activity of the combination.

Examples of such diluents that are especially useful for injectable formulations are water, the various saline, organic or inorganic salt solutions, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may include additives such as other carriers; adjuvants; or non-toxic, non-therapeutic, non-immunogenic stabilizers and the like.

Furthermore, excipients can be included in the compositions disclosed. Examples include but are not limited to cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Any pharmacologically acceptable buffer may be used, such as, tris or phosphate buffers. Effective amounts of diluents, additives, and excipients are those amounts that are

effective to obtain a pharmaceutically acceptable formulation in terms of solubility, biological activity, etc.

The pharmaceutical compositions comprising the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein may be incorporated into a microsphere. The compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein can be loaded into albumin microspheres, from which it is possible to recover such microspheres in a dry powder for nasal administration. Other materials suitable for the preparation of microspheres include agar, alginate, chitosan, starch, hydroxyethyl starch, albumin, agarose, dextran, hyaluronic acid, gelatin, collagen, and casein. The microspheres can be produced by various processes known to the person skilled in the art such as a spray drying process or an emulsification process.

For example, albumin microspheres can be prepared by adding rabbit serum albumin in phosphate buffer to olive oil with stirring to produce water in oil emulsion. Glutaraldehyde solution is then added to the emulsion and the emulsion stirred to cross-link the albumin. The microspheres can then be isolated by centrifugation, the oil removed and the spheres washed, e. g., with petroleum ether followed by ethanol. Finally, the microspheres can be sieved and collected and dried by filtration.

Starch microspheres can be prepared by adding a warm aqueous starch solution, e. g. of potato starch, to a heated solution of polyethylene glycol in water with stirring to form an emulsion. When the two-phase system has formed (with the starch solution as the inner phase) the mixture is then cooled to room temperature under continued stirring whereupon the inner phase is converted into gel particles. These particles are then filtered off at room temperature and slurred in a solvent such as ethanol, after which the particles are again filtered off and laid to dry in air. The microspheres can be hardened by well-known cross-linking procedures such as heat treatment or by using chemical cross-linking agents. Suitable agents include dialdehydes, including glyoxal, malondialdehyde, succinicaldehyde, adipaldehyde, glutaraldehyde and phthalaldehyde, diketones such as butadione, epichlorohydrin, polyphosphate, and borate. Dialdehydes are used to cross-link proteins such as albumin by interaction with amino groups, and diketones form schiff bases with amino groups. Epichlorohydrin activates compounds with nucleophiles such as amino or hydroxyl to an epoxide derivative.

Another preferred embodiment of the invention is the dosage scheme of the compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, and of the compositions comprising said compounds, as described above herein. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for subjects, e. g., mammalian subjects, e. g. humans, dogs, cats, and rodents, each unit containing a predetermined quantity of active material calculated to produce the desired pharmaceutical effect in association with the required pharmaceutical diluent, carrier or vehicle. The specifications for the unit dosage forms of this invention are dictated by and dependent on (a) the unique characteristics of the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, disclosed above herein and the particular effect to be achieved and (b) the limitations inherent in the art of compounding said compound of Formula (**I**) for use in humans and animals. Examples of unit dosage forms are tablets, capsules, pills, powder packets, wafers, suppositories, granules, cachets, teaspoonfuls, tablespoonfuls, dropperfuls, ampoules, vials, aerosols with metered discharges, segregated multiples of any of the foregoing, and other forms as herein described. The compositions disclosed herein can be included in kits, which can contain one or more-unit dosage forms of the composition and instructions for use to treat one or more of the diseases described herein.

Slow or extended-release delivery systems, including any of a number of biopolymers (biological-based systems), systems employing liposomes, colloids, resins, and other polymeric delivery systems or compartmentalized reservoirs, can be utilized with the compositions described herein to provide a continuous or long-term source of therapeutic compound. Such slow release systems are applicable to formulations for delivery via topical, intraocular, oral, and parenteral routes.

An effective amount of the compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, as described above herein, is comprised in the compositions. The dosage of compounds used in accordance with the invention varies depending on the compound and the condition being treated for example the age, weight, and clinical condition of the recipient patient. Other factors include: the route of administration, the patient, the patient's medical history, the severity of the disease process, and the potency of the particular compound. The dose should be sufficient to ameliorate symptoms or signs of the disease treated without producing unacceptable toxicity to the patient. In general, an effective amount of the compound is that which

provides either subjective relief of symptoms or an objectively identifiable improvement as noted by the clinician or other qualified observer.

One embodiment disclosed herein refers to a method of use of the compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, in the treatment of conditions or diseases responsive to the activation of the HIF pathway.

Said compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, show capacity to bind PHD2, stabilize HIF-1α and HIF-2α proteins, activate the HIF pathway in different cell types, induce angiogenesis in human endothelial vascular cell, show neuroprotective activity *in vitro* and *in vivo*, antidiabetic activity and reduce the levels of lipids *in vivo*, and increase the plasma levels of Erythropoietin *in vivo*. Said compounds of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, act in a selective manner and do not induce Nrf2 activation, NF-κB inhibition, STAT3 inhibition, and TGR5 activation, which are known activities for their natural triterpenoid precursors.

Another embodiment disclosed herein refers to a method of use of the compounds of Formula (**I**) for treating diseases responsive to the activation of the HIF pathway such as IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases. In a preferred embodiment said diseases are selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, organ transplantation, acute kidney injury or arterial diseases.

Another embodiment disclosed herein refers to a method of use of the compounds of Formula (**I**) in the treatment of a condition or disease responsive to the activation of the HIF pathway, selected from to stroke, cerebral palsy, traumatic injuries or neurodegenerative diseases.

One embodiment disclosed herein refers to a method of treatment of a condition or disease responsive to the activation of the HIF pathway selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases, said method comprising administering an effective amount of a compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers,

polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, as described above herein, to an individual in need of said treatment. In a preferred embodiment said diseases are selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, organ transplantation, acute kidney injury or arterial diseases.

Another embodiment disclosed herein refers to a method of treatment of a condition or disease responsive to the activation of the HIF pathway, said method comprising administering an effective amount of a compound of Formula (**I**) or analogs, derivatives, tautomeric forms, isomers, stereoisomers, polymorphs, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, as described above herein, to an individual in need of said treatment.

In one embodiment disclosed herein, condition or disease responsive to the activation of the HIF pathway include but are not limited to stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases. Examples of neurodegenerative diseases include but are not limited to Alzheimer disease, Parkinson disease, Huntington disease or Multiple Sclerosis.

Brief description of the figures

Figure 1. Top scored conformation of betulinic acid (Fig. 1A) and compound **VII** (Fig. 1B) bound to PHD2 (PDB 4BQW).

Figure 2. HIF-1α transactivation assays in HaCaT-EPO Luc keratinocyte cells.

Hypoximimetic effects of DFX and compounds **II** to **XV** in HaCaT-EPO-Luc cells. The concentration of the tested compound (μM) is shown at the x-axis and the percentage of HIF-1α activation is shown at the y-axis. This figure shows the effect of DFX versus compounds (Figure 2A) **II**, **III**, **IV**, **V, VI, VII, XI,** (Figure 2B) **VIII, IX, X, XII, XIII, XIV and XV** on EPO-luc activity considering the induction mediated by DFX (150 μM) as 100% activation over untreated cells.

Figure 3. HIF-1α stabilization in oligodendrocytes.

Stimulation of human oligodendrocyte MO13.3 cells for 3 hours with either 150 μDFX or 10 μM of Olenaolic acid (OA), compounds II, III, IV, V, VI (Fig. 3A), betulinic acid (BA), compounds VII, VIII, IX, X, XI (Fig. 3B), ursolic acid (UA), maslinic acid (MA), compounds XII, XIII, XIV and XV (Fig. 3C) to determine the expression of HIF-1α and β-actin by Western blots.

Figure 4. HIF-1α stabilization in 293T cells.

Stimulation of human Embryonic Kidney 293 cells with increasing concentrations of compound **VII** or with DFX (150 μM) during 3 h. The steady state levels of the proteins HIF-1α, PHD1, PHD2 and β-actin were determined by Western blots.

Figure 5. HIF-2α stabilization in hIPCs.

Stimulation of human Islet-Derived Precursor Cells (hIPCs) with increasing concentrations of compound **VII** or with DFX (150 μM) during 3 h, and the expression of HIF-2α, PHD3 and β-actin determined by Western blots.

Figure 6. Compound VII induces angiogenesis.

Measurements of endothelial cell tube formation as a model of angiogenesis in green fluorescent Human endothelial vascular cells (HUVEC) co-cultured with primary fibroblasts and stimulated separately with compound **VII** (1 μM), rhFGF (10 ng/ml) and VEGFA (10 ng/ml) for 7 days. Values represent the mean ± SEM (n=3).

Figure 7. Influence of compound VII on erythropoietin (EPO) in vivo.

EPO in plasma measured using a mouse EPO ELISA kit in C57BL/6 male mice administered with compound **VII** (30 mg/kg or 60 mg/kg) or betulinic acid (BA) (60 mg/kg) intraperitoneally. Control group did not receive any treatment. Data are expressed as mean ± SEM (n=3).

Figure 8. Compound VII abrogates 3-NP toxicity in Q7 and Q11 striatal cells. Q7 and Q111 Striatal cells were pretreated with increasing concentrations of compound **VII** for 6 h and then exposed to 3-NP (10 mM) for an additional 24 h, after which YOYO-1 staining was used to investigate cell death. Cell death was determined using the IncuCyte HD software and the treatment with 3-NP alone in Q111 cells was considered as 100 % of cell death.

Figure 9. Behavioral score after 3NP intoxication.

Intoxicated mice with 3-Nitropropionic acid (3-NP) were subjected to behavioral tests for determining their neurological status after the treatment with **compound VII** (30 mg/kg) and Betulinic acid (BA) (30 mg/kg) versus a control of non-intoxicated mice. Hind limb clasping, Locomotor activity, Hind limb dystonia and Truncal Dystonia were rated from 0 to 2 based on severity: a score of 0 typically indicates normal function and 2 seriously affected. Values are expressed as means ± SEM for 6 animals *per* group.

Figure 10. Effect of compound VII on neuronal loss.

Intoxicated mice with 3-Nitropropionic acid (3-NP) for determining degeneration in the striatum including Control (non-intoxicated mice), 3NP, 3NP+BA and 3NP + Compound **VII)**. Quantification of Nissl-positive cells in the mouse striatum. Total average number of neurons (100× magnification) is shown. Values are expressed as means ± SEM for 6 animals per group. Data were subjected to one-way analysis of variance followed by the Student–Newman–Keuls test. ***$P < 0.001$ when comparing the control group with the 3NP and control group. $^{\#}P < 0.05$ when comparing the 3NP group with the 3NP + compound **VII** group

Figure 11. Effect of compound VII on microgliosis ($Iba1^+$) and astrogliosis ($GFAP^+$) induced by 3NP

Intoxicated mice with 3-Nitropropionic acid (3-NP) for determining microglia activation and astrogliosis including Control (non-intoxicated mice), 3NP, 3NP+BA and 3NP + Compound **VII)**. Iba-1 and glial fibrillary acidic protein (GFAP) expression were determined by immunostaining of brain sections through the different group of mice and quantification of the different markers was performed with Image J software. Total average number of microglia ($Iba1^+$) and astrocytes ($GFAP^+$) is shown.

Figure 12. Compound VII reduces the expression on inflammatory marker mRNAs in the striatum.

Gene expression of inflammatory markers including COX-2 (Figure 12D), IL-1β (Figure 12B), IL-6 (Figure 12A) and iNOS (Figure 12C), was down regulated in 3NP+compound **VII** (30 mg/kg) treated mice compared with 3NP+Vehicle mice. Betulinic acid (BA) treatment (30 mg/kg) also inhibited the expression of inflammatory markers. Expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Values are expressed as means ± SEM for 6 animals *per* group.

Figure 13. Effect of compound VII on body weight, fat mass and adiposity in mice subjected to high fat diet (HFD animals).

Mice were fed with HFD for 13 weeks and the treated daily with compound VII (30 mg/Kg) for 21 days and the effect on body weight was monitored every 3-4 days (Figure 13A). % Lean mass (Figure 13B) is calculated as the proportion of entire weight of the body without the proportion due to fat. % Fat mass (Figure 13C) is calculated as the proportion of fat to the total body weight. Fat mass and the percentage of adiposity was calculated at the week 15. Body composition was assessed by Magnetic Resonance Imaging (MRI).Values are expressed as means ± SEM for 10 animals *per* group.

Figure 14. Compound VII improves glucose tolerance (GTT) in HFD animals.
GTT of the HFD-fed mice after the 3-week vehicle (Control, CD) or compound VII administration (30 mg/Kg). The sum of the trapezoidal areas between the 0, 15, 30, 45, 60, 90 and 120 min time points corresponding to each animal were summed to obtain the area under the curve (AUC). The relative area values are expressed as a percentage relative to the average AUC of the vehicle cohort, which is defined as 100%. Values are expressed as mean ± S.E.M (n=6).

Figure 15. Compound VII prevents hypertriglyceridemia in HFD animals.
Plasma triglycerides levels in control and HFD-fed mice after the 3-week vehicle (Control, CD) or compound VII administration (30 mg/Kg). Values are expressed as mean ± S.E.M (n=6).

Examples

The examples of the present invention described below aim to illustrate its preferred embodiments without limiting its scope of protection.

Example 1. Synthesis of the triterpenoid derivatives of Formula (I) and of comparative compound XX.

General synthesis of the *N*-hydroxy-triterpen-28-amide derivatives of Formula (I) (hydroxamate formation):

To an ice-cold solution of a triterpenic acid precursor (1 eq/mol) in dry dichloromethane (DCM), oxalyl chloride (6 eq/mol) was added dropwise, and the mixture was heated at 40°C for 1,5 hours. The solvent was removed in vacuum, the residue was dissolved in dry pyridine or N,N-diisopropylethylamine (DiPEA), and hydroxylammonium chloride (6 eq/mol) were added. The reaction was heated at 40°C for 3 hours, quenched with 2 N H_2SO_4 sol. and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude compound was purified over silica gel.

Known compounds are identified when possible with means of their CAS number.

(3β) 3-Hydroxy-*N*-hydroxy-olean-12-en-28-amide (compound II)

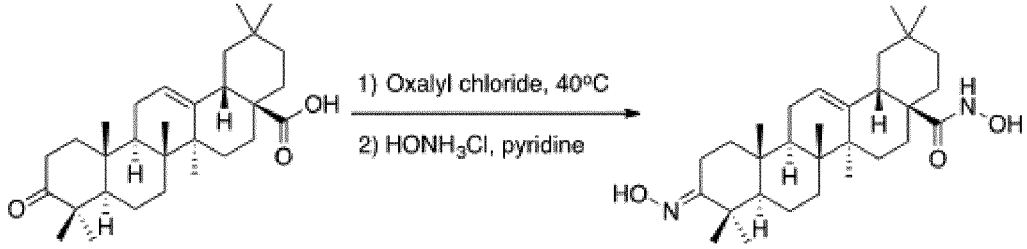
CAS num.: 1854922-22-7

Off-white solid (60%). ^{1}H NMR (300 MHz, $CDCl_3$): d: ^{1}H NMR (300 MHz, $CDCl_3$): d = 5.41 (*brt*, 1H, H-12), 3.19 (*dd*, *J* = 10.0, 4.89 Hz, 1H), 2.44 (*d*, *J* = 10.8 Hz, 1H), 2.05–1.86 (*m*, 3H), 1.16 (*s*, 3H), 0.98 (*s*, 3H), 0.92 (*s*, 3H), 0.90 (*s*, 3H), 0.88 (*s*, 3H), 0.81 (*s*, 3H), 0.78 (*s*, 3H) (only

readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$): d = 176.7, 144.9, 123.9, 78.9, 55.2, 47.6, 46.4, 45.5, 42.0, 40.8, 39.5, 38.8, 38.6, 37.0, 34.0, 32.9, 32.3, 32.0, 30.7, 28.1, 27.3, 27.2, 25.84, 23.7, 23.6, 23.5, 18.3, 16.7, 15.6, 15.4.

3-Hydroxyimino-*N*-hydroxy-olean-12-en-28-amide (compound III)

Compound **III** was obtained according to the general synthetic scheme described above herein (hydroxamate formation), starting from the known precursor CAS num. 17990-42-0:



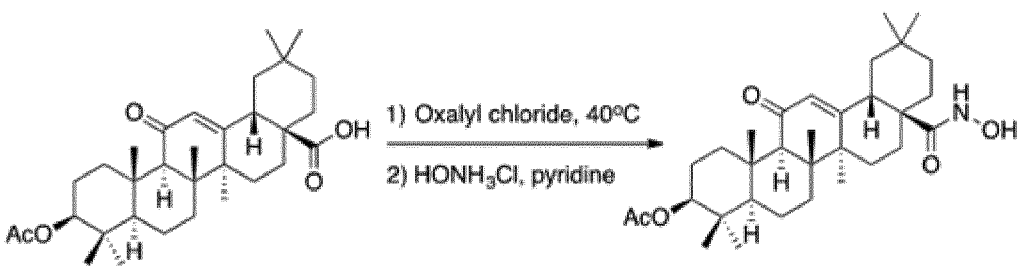
17990-42-0 (III)

The oxime formation featured in position C of compound **III** was carried out as well as the hydroxamate formation during the same general synthetic path.

Off-white solid (70%). ^1H NMR (300 MHz, $CDCl_3$): d = 5.43 (*brt*, 1H), 3.07 (*bdt*, *J*=14.9, 1H), 2.46 (*m*, 1H), 1.98 (*m*, 3H), 1.13 (*s*, 10H), 1.07 (*s*, 6H), 1.05 (*s*, 3H), 0.86 (*s*, 6H), 0.82 (*s*, 3H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) d = 176.7, 167.5, 144.9, 123.8, 55.7, 47.1, 46.2, 45.5, 42.0, 40.8, 40.3, 39.4, 38.4, 37.0, 33.9, 32.9, 31.9, 30.7, 29.7, 29.2, 27.2, 25.7, 25.5, 23.7, 23.5, 23.4, 19.0, 17.3, 16.7, 14.9.

(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide (compound V)

Compound **V** was obtained according to the general synthetic scheme described above herein (hydroxamate formation), from the known precursor CAS num. 14605-17-5:

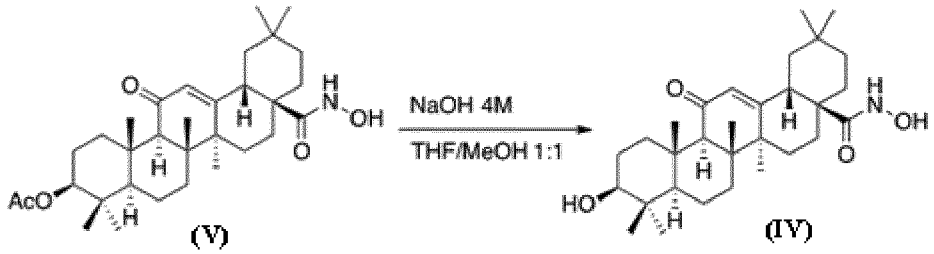


14605-17-5 (V)

Pale yellow solid (75%).[1]H NMR (300 MHz, $CDCl_3$): d = 5.65 (*s*, 1H), 4.47 (*dd, J* = 4.8 *J* = 11.0 Hz, 1H), 3.31 (*m*, 1H), 2.76 (*t, J* = 12.5 Hz, 2H), 2.32 (*s*, 1H), 2.00 (*s*, 3H), 1.12 (*s*, 3H), 0.91 (*s*, 3H), 0.88 (*s*, 6H), 0.82 (*s*, 9H) (only readily peaks are reported); [13]C NMR (75 MHz, $CDCl_3$) d = 200.5, 174.4, 171.2, 168.4, 127.8, 80.7, 61.9, 55.0, 45.3, 44.8, 43.6, 43.0, 40.7, 38.7, 38.0, 37.1, 33.8, 32.8, 32.6, 32.1, 30.7, 28.1, 27.3, 23.6, 23.5, 23.4, 23.1, 21.3, 19.0, 17.3, 16.7, 16.3.

(3β) 3-Hydroxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide (compound IV)

To obtain compound **IV**, the deacetylation of compound **V** was carried out as follows:



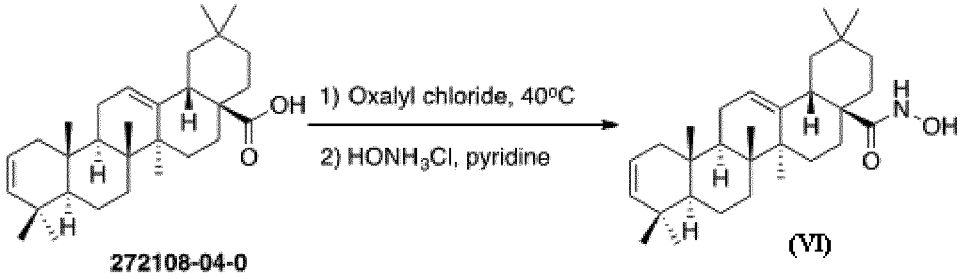
(V) (IV)

To a solution of compound V, (3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide (1 eq/mol) in THF/MeOH 1:1 was added NaOH 4N (50 eq/mol). The mixture was heated at 40°C overnight, quenched with H_2SO_4 sol. 2N and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude compound was purified over silica gel.

Pale yellow solid (65%). [1]H NMR (300 MHz, $CDCl_3$): d = 5.67 (*s*, 1H), 3.20 (*t, J* = 6.1 Hz, 1H), 2.74 (*d, J* = 12.2 Hz, 2H), 2.32 (*s*, 1H), 2.10-2.02 (*m*, 1H), 1.18 (*s*, 3H), 0.96 (*s*, 3H), 0.94 (*s*, 3H), 0.90 (*s*, 9H), 0.77 (*s*, 3H) (only readily peaks are reported); [13]C NMR (75 MHz, $CDCl_3$) d = 200.2, 175.8, 167.9, 128.0, 78.8, 62.1, 55.0, 45.2, 44.7, 43.6, 40.9, 39.2, 37.3, 33.7, 32.8, 32.7, 32.1, 30.7, 29.7, 28.1, 27.4, 27.3, 23.7, 23.4, 23.3, 19.0, 17.5, 16.2, 15.6, 14.2.

Oleana-2,12-dien-*N*-hydroxy-28-amide (compound VI)

Compound **VI** was obtained following the general synthetic scheme described above herein (hydroxamate formation), starting from the known precursor CAS num. 272108-04-0:



272108-04-0 (VI)

Yellow oil (48%). ^{1}H NMR (300 MHz, $CDCl_3$): d = 5.43-5.32 (*m*, 3H), 2.44 (*d*, *J* = 11.3 Hz, 1H), 1.14 (*s*, 3H), 0,97 (*s*, 6H), 0,87 (*s*, 12H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) d = 176.4, 144.5, 138.0, 124.1, 121.3, 51.9, 46.4, 46.1, 45.5, 42.1, 41.0, 40.7, 39.5, 36.1, 34.5, 34.0, 33.0, 31.9, 31.8, 31.6, 30.7, 27.2, 25.9, 25.7, 23.8, 23.5, 22.9, 19.6, 16.3, 15.6.

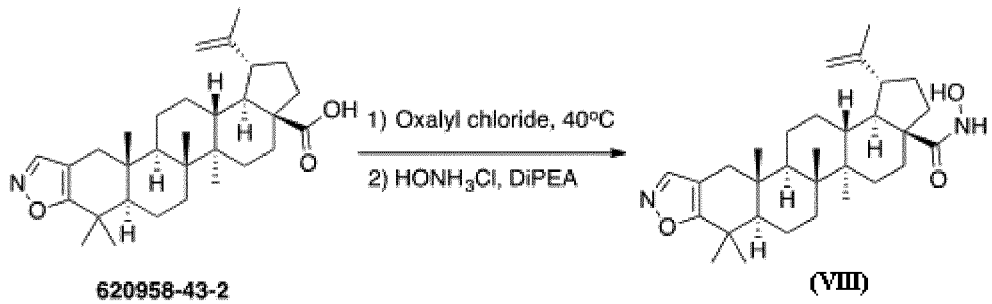
(3β) 3-Hydroxy-*N*-hydroxy-lup-20(29)-en-28-amide (compound VII)

CAS num.: 1822375-07-4

Off-white solid (55%). ^{1}H NMR (300 MHz, $CDCl_3$): d: 4.73 (s, 1H), 4.60 (s, 1H), 3.20-3.15 (m, 1H), 3.05 (ddd, J=11.4, 6, 4.49 Hz, 1H), 1.67 (s, 3H), 0.95 (s, 6H), 0.92 (s, 3H), 0.80 (s, 3H), 0.74 (s, 3H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$): d: 175.2, 150.9, 108.5, 78.4, 55.7, 54.3, 50.8, 50.7, 46.7, 42.3, 40.7, 38.7, 38.2, 37.8, 37.1, 36.9, 34.3, 32.6, 30.7, 29.3, 27.8, 26.9, 25.5, 20.8, 19.2, 18.2, 16.0, 15.9, 15.2, 14.5.

Lup-2-eno[2,3-d]isoxazol-*N*-hydroxy-28-amide (compound VIII)

Compound **VIII** was obtained following the general synthetic scheme described above herein (hydroxamate formation), starting from the known precursor CAS num. 620958-43-2:

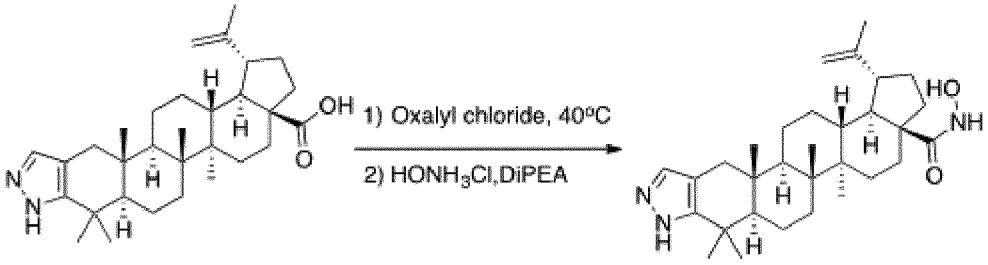


620958-43-2 (VIII)

White solid. ^{1}H NMR (300 MHz, CD_3OD): d = 10.36 (*s*, 1H, NH), 8.32 (*s*, 1H, OH), 8.26 (*s*, 1H), 4.67 (*s*, 1H), 4.55 (*s*, 1H), 3.00 (*t*, *J* = 9.3 Hz, 1H), 2.61 (*t*, *J* = 12.0 Hz, 1H), 1.64 (*s*, 3H), 1.40 (*s*, 3H), 1.22 (*s*, 3H), 1.11 (*s*, 3H), 0.93 (*s*, 3H), 0.74 (*s*, 3H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) d = 172.6, 151.3, 151.0, 109.9, 109.4, 54.0, 53.3, 50.6, 49.0, 48.9, 46.7, 42.4, 38.9, 37.3, 35.6, 34.8, 33.4, 32.6, 30.9, 29.0, 25.7, 21.7, 19.5, 18.7, 16.4, 16.2, 14.8.

1'H-Lup-20(29)-eno[3,2-c]pyrazol-*N*-hydroxy-28-amide (compound IX)

Compound **IX** was obtained according to the general synthetic scheme described above herein (hydroxamate formation), starting from the known precursor CAS num. 1334386-31-0:

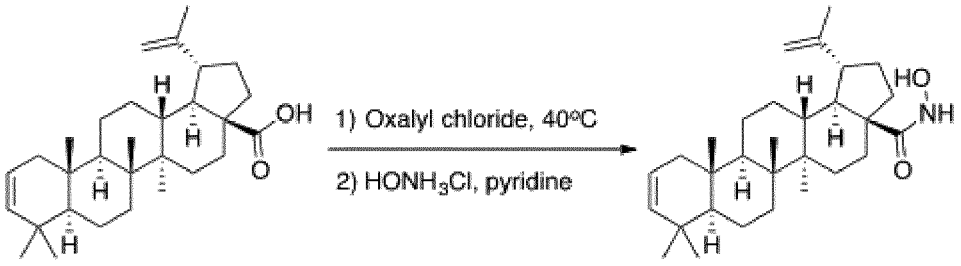


1334386-31-0 (IX)

Pale yellow solid. ^{1}H NMR (300 MHz, $(CD_3)_2CO$): d = 7.17 (*s*, 1H), 4.72 (*s*, 1H), 4.58 (*s*, 1H), 3.20-3.13 (*m*, 1H), 2.69-2.61 (*m*, 1H), 1.69 (*s*, 3H), 1.28 (*s*, 3H), 1.18 (*s*, 3H), 1.02 (*s*, 3H), 0.98 (*s*, 3H), 0.80 (*s*, 3H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $(CD_3)_2CO$) d = 172.4, 151.0, 149.1, 132.8, 111.9, 109.0, 59.7, 50.0, 53.7, 50.5, 49.2, 46.8, 42.2, 40.7, 38.6, 37.9, 37.7, 36.6, 33.5, 33.4, 32.3, 30.8, 30.6, 25.7, 23.3, 21.4, 19.1, 18.7, 15.6, 14.2, 13.7.

Lupa-2,20(29)-dien-*N*-hydroxy-28-amide (compound X)

Compound **X** was obtained according to the general synthetic scheme described above herein (hydroxamate formation), starting from the known precursor CAS num. 173106-19-9:

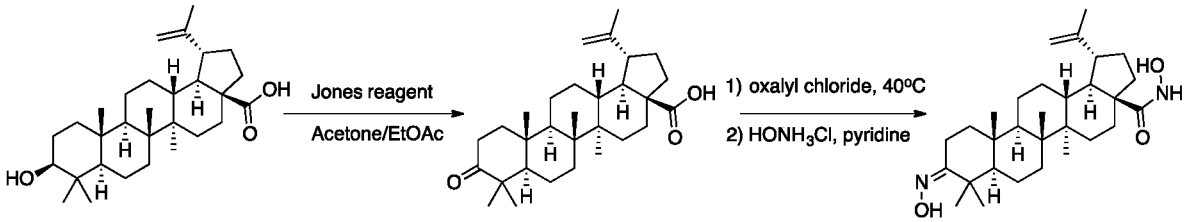


173106-19-9 (X)

Pale yellow solid (54%) ^{1}H NMR (300 MHz, $CDCl_3$): d = 5.38-5.28 (*m*, 2H), 4.69 (*s*, 1H), 4.56 (*s*, 1H), 3.01 (*t*, *J* = 10.7 Hz, 1H), 2.37 (*t*, *J* = 12.1 Hz, 1H), 1.63 (*s*, 3H), 1.21 (*s*, 3H), 0,93 (*s*, 3H), 0,89 (*s*, 3H), 0,82 (*s*, 3H), 0,81 (*s*, 3H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) d = 175.0, 150.5, 137.9, 121.6, 109.65, 54.3, 52.1, 50.4, 49.2, 42.3, 40.8, 38.4, 37.9, 36.4, 34.6, 33.5, 32.8, 31.7, 30.9, 30.8, 29.7, 29.3, 25.6, 22.6, 19.5, 16.4, 15.8, 14.6, 14.5, 14.3.

3-Hydroxyimino-*N*-hydroxy-lup-20(29)-en-28-amide – step (b) - (compound XI)

Prior to the general synthetic scheme described above herein (hydroxamate formation), the conversion of the hydroxyl group of the C-3 position of betulinic acid into a carbonyl group was carried out as follows:



(XVI) (XI)

To a solution of betulinic acid (1 gr, 2.19 mmol) in acetone/EtOAc 5:5 (10 mL) was added Jones reagent until the disappearance of the starting material (control by TLC). The reaction was washed with brine and extracted with EtOAc. The organic phases were dried over Na_2SO_4 and evaporated under vacuum and the crude was purified over silica gel (PE/EtOAc 9:1), 3-Oxo-lup-20(29)-en-28-acid (compound **XVI**; CAS num.: 4481-62-3) (89%) as an off-white solid.

Compound **XVI** was then used to carry out the hydroxamate formation following the general synthetic scheme described above herein to obtain compound **XI**.

Off-white solid (65%). ^{1}H NMR (300 MHz, $CDCl_3$): d = 4.73 (*s*, 1H), 4.61 (*t*, *J* = 6.1 Hz, 1H), 3.04-3.00 (*m*, 2H), 2.32 (*s*, 1H), 1.68 (*s*, 3H), 1.24 (*s*, 6H), 1.22 (*s*, 3H), 1.12 (*s*, 3H), 0.97 (*s*, 3H), 0.96 (*s*, 3H), 0.91 (*s*, 3H) (only readily peaks are reported); 177.3, 167.3, 150.5, 109.6, 55.6, 55.0, 50.3, 50.2, 46.8, 42.5, 40.8, 40.2, 38.7, 38.2, 37.9, 37.2, 34.0, 33.3, 30.8, 29.4, 27.4, 25.6, 22.9, 21.5, 21.2, 19.4, 19.1, 16.1, 15.8, 14.6.

(3β) 3-Hydroxy-*N*-hydroxy-urs-12-en-28-amide (compound XII)

CAS num.: 915415-61-1

Off-white solid (55%). ^{1}H NMR (300 MHz, $CDCl_3$): d = 5.34 (*brt*, 1H, H-12), 3.18 (*dd*, *J* = 9.5, 3.9 Hz, 1H), 2.09–1.92 (*m*, 3H), 1.06 (*s*, 3H), 0.94 (*s*, 3H), 0.90 (*d*, *J* = 3.2 Hz, 3H), 0.87 (*d*, *J* = 7.8 Hz, 3H), 0.80 (*s*, 3H), 0.78 (*s*, 3H), 0.75 (*s*, 3H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$): d = 176.8, 140.1, 126.6, 126.6, 79.0, 55.1, 52.2, 47.5, 42.4, 39.5, 39.5, 39.0, 38.8, 36.9, 28.1, 25.7, 23.3, 21.2, 17.2, 16.7, 15.7, 15.5.

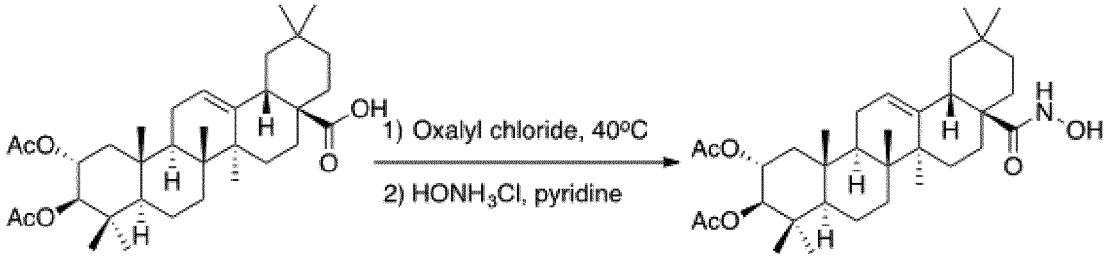
3 Hydroxyimino-*N*-hydroxy-urs-12-en-28-amide (compound XIII)

Compound disclosed in patent CN102180939. Said compound was prepared from ursolic acid carrying out an analogous synthesis to compound **XI**, wherein in a first step the conversion of the C-3 hydroxyl to carbonyl using Jones reagent is carried out, followed by the hydroxamate formation according to the general synthetic scheme described above herein to obtain compound **XIII**.

Pale yellow solid (70%). ^{1}H NMR (300 MHz, $CDCl_3$): d = 5.40 (*brt*, 1H), 3.07 (*bdt*, *J*=15.6, 1H), 2.12 (*m*, 1H), 1.24 (*s*, 3H), 1.15 (*s*, 3H), 1.08 (*s*, 3H), 1.06 (*s*, 3H), 1.03 (*s*, 3H), 0.94 (*s*, 3H), 0.81 (*s*, 3H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) d = 177.3, 167.7, 140.6, 126.5, 55.7, 52.1, 47.0, 42.5, 40.2, 39.6, 39.4, 39.0, 38.5, 37.0, 36.7, 32.2, 30.6, 29.7, 27.7, 27.4, 24.8, 23.5, 23.4, 23.3, 21.1, 19.0, 17.3, 17.2, 16.8, 15.1.

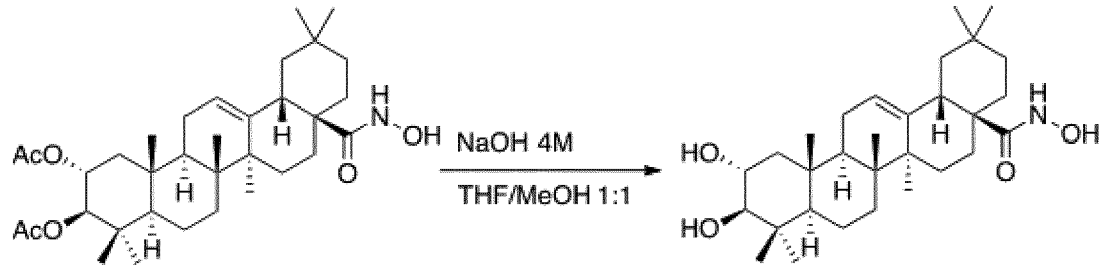
(2α,3β) 2,3-Dihydroxy-*N*-hydroxy-olean-12-en-28-amide (compound XIV)

Compound **XIV** was obtained according to the general synthetic scheme described above herein (hydroxamate formation), starting from the known precursor CAS num.: 6089-92-5



6089-92-5 (XIX)

Compound **XIX** was deacetylated to obtain compound **XIV**:

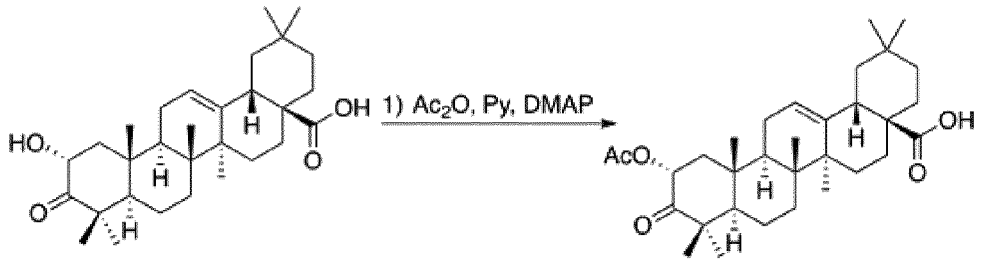


(XIX) (XIV)

Off-white solid (45%). ^{1}H NMR (300 MHz, $CDCl_3$): d = 5.44 (*brt*, 1H), 3.72-3.61 (*m*, 1H), 2.99 (*d*, *J* = 9.5 Hz, 1H), 2.45 (*d*, *J* = 12.2 Hz, 1H), 1.15 (*s*, 3H), 1.02 (*s*, 3H), 0.98 (*s*, 3H), 0.90 (*s*, 3H), 0.87 (*s*, 3H), 0.82 (*s*, 3H), 0.78 (*s*, 3H) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$): d = 175.9, 144.0, 123.6, 83.7, 68.2, 55.0, 47.3, 46.2, 46.1, 45.6, 41.2, 41.0, 39.0, 38.7, 37.8, 33.6, 32.6, 32.4, 32.3, 31.4, 28.5, 27.4, 27.3, 23.2, 23.1, 22.8, 18.6, 16.3, 16.5, 16.1.

2-Hydroxy-3-oxo-oleana-1,12-dien-*N*-hydroxy-28-amide (compound XV)

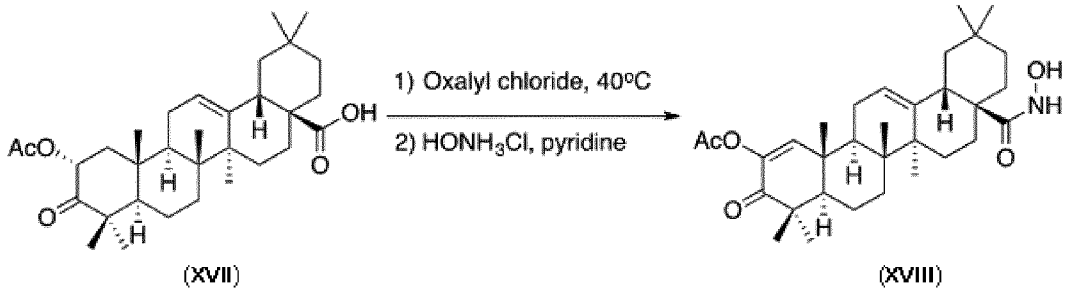
Prior to the general synthetic scheme described above herein (hydroxamate formation), the acetylation of the hydroxyl group of the C-2 position of a known maslinic acid derivative with CAS num. 1382923-75-2 was carried out as follows:



1382923-75-2 (XVII)

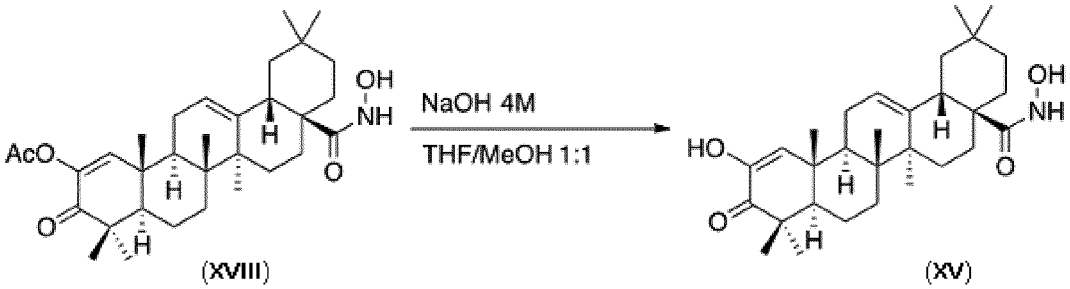
To a solution of the known maslinic acid derivative (1 eq/mol) in dry pyridine (10 mL per gr of acid) were sequentially added acetic anhydride (2 eq/mol) and DMAP (0.1 eq/mol). The reaction is stirred at room temperature until the disappearance of the starting material (control by TLC), quenched with methanol, diluted with H_2SO_4 sol. 2N and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4 and evaporated under vacuum to give compound **XVII** without further purification.

Compound **XVII** was then used to carry out the hydroxamate conversion following the general synthetic scheme described above herein as follows:



(XVII) (XVIII)

Compound **XVII** was then deacetylated to obtain compound **XV** as follows:

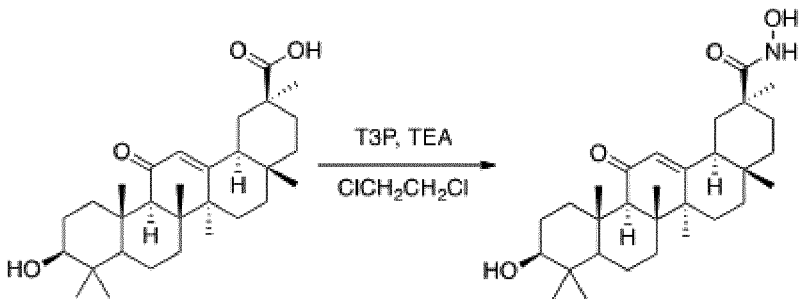


(XVIII) (XV)

To a solution of compound **XVII** (1 eq/mol) in THF/MeOH 1:1 was added NaOH 4N (50 eq/mol). The mixture was heated at 40°C overnight, quenched with H_2SO_4 sol. 2N and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude compound was purified over silica gel.

Pale yellow solid (69%). ^{1}H NMR (300 MHz, $CDCl_3$): d = 6.31 (*s*, 1H), 5.47 (*brt*, 1H), 2.46 (*d*, *J* = 11.3 Hz, 1H), 1.20 (*s*, 3H), 1.19 (*s*, 3H), 1.13 (*s*, 3H), 1.10 (*s*, 3H), 0.88 (*s*, 3H), 0.86 (*s*, 6H), (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) d = 201.0, 176.4, 145.2, 143.8, 127.8, 123.2, 53.7, 46.1, 45.5, 44.0, 43.1, 42.4, 40.9, 40.1, 38.4, 33.9, 32.9, 32.0, 30.7, 27.1, 27.0, 25.8, 23.6, 23.4, 21.9, 20.8, 19.6, 18.7, 17.1, 14.2.

As a comparative compound **XX** was synthesized from glycyrrhetinic acid (GA) by using a T3P/triethylamine protocol without the need of protecting groups (Ech-Chahad et al., Tetrahedron Letters 2005, 46: 5113–5115).



GA (XX)

***In silico* assays**

Example 2. Calculation of PHD2-binding affinity *in silico*.

Molecular structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) or designed with MarvinSketch (ChemAxon, Cambridge, MA). The receptor model used was the PDB reference 4BQW. Binding properties were calculated by using the AutoDock4 (Morris et al., J. Comp. Chem. 2009 Dec;30(16)2785-91) and the Vina software (Trott and Olson, J. Comp. Chem. 2010 Jan 30;31(2):455-61) with the virtual screening tool PyRx (Wolf LK. Chem. & Eng. News 2009, 87) The search space for the docking, around the receptor molecule surface, was set according to previous findings about several binding sites for different PHD2 ligands (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025).

Once analysis has been performed, AutoDock Vina provides the estimated binding affinity value, which is the sum of the intermolecular energy, due to the interaction between both molecules, and the torsional free-energy penalty, due to the conformation adopted by these molecules to properly fit the interaction surface. A negative value indicates that bond is thermodynamically stable, whereas a positive value means instability.

Table I shows the PHD2-binding energy (Kcal/mol) for the compounds described in the present invention and also for positive (IOX-2) and negative (glycyrrethinic acid) controls. Mean values from AutoDock-Vina using the structure 4BQW (from Protein Data Bank) are shown. Average values from three independent experiments are shown.

COMPOUND	B.E. AutoDock (Kcal/mol)
Oleanolic acid	-6.35
Compound II	-6.33
Compound III	-3.30
Compound IV	-6.41
Compound V	-3.35
Compound VI	-5.27
Betulinic acid	-4.76
Compound VII	-6.55
Compound VIII	-5.77
Compound IX	-4.33
Compound X	-7.08
Compound XI	-6.09
Ursolic acid	-5.74
Compound XII	-6.01
Compound XIII	-5.45
Maslinic acid	-3.20
Compound XIV	-4.30
Compound XV	-3.57
IOX 2	-9.05
Glycyrrethinic acid	-1,24

Table I. Energy binding of triterpenoids and derivatives to PDH2. PHD2-binding energy (Kcal/mol). Mean values from *AutoDock/Vina* using the structure 4BQW (from *Protein Data Bank*) are shown. Average values from three independent experiments are shown.

Search space was restricted to a 300 Å volume around residues H313, D315, H374, R383, Y303, Y310, Y329, I327, I256 and M299, proposed as main binding sites by building a grid surpassing these space in 10 Amstrong along the three axes by (Rabinowitz MH, J. Med. Chem. 2013 Dec 12;56(23):9369-4025) (**Fig. 1**). The resulting binding energy found for Compound **VII** was -6.55 Kcal/mol, Ki 15.74 uM (at 298.15ºK), intermolecular energy -7.38 Kcal/mol and root mean square deviation from atomic position 0.0 Å, thus improving the binding features found for its natural precursor betulinic acid, with a binding energy of -4.76 Kcal/mol.

***In vitro* and *in vivo* assays**

Example 3. Selective induction of HIF-1α activity

To investigate the biological activities of the different compounds, HIF-1α transactivation assays were performed either in NIH-3T3-EPO-Luc cells (**Table II**) or in HaCaT-EPO-luc cells (**Fig. 2**). The NIH3T3-EPO-luc and HaCaT-EPO-luc cells have been stably transfected with the plasmid Epo-Luc plasmid. The EPO-Hypoxia Response Element (HRE)-luciferase reporter plasmid contains three copies of the HRE consensus sequence from the promoter of the erythropoietin gene fused to the luciferase gene. NIH3T3-EPO-luc cells were maintained at 37 ºC in a humidified atmosphere containing 5% CO_2 in DMEM supplemented with 10% fetal calf serum (FBS), and 1% (v/v) penicillin/streptomycin. DFX was purchased from Sigma-Aldrich (USA). Cells ($1x10^4$/well in 96 well plates) were seeded the day before the assay. The next day, the cells were stimulated either with increasing concentrations of either Oleanolic acid (OA), Betulinic acid (BA), Ursolic acid (UA), Maslinic acid (MA) Glycyrrethinic acid (GA), compounds of Formula (**I**), **II** to **XV,** or comparative compounds **XX** and **CDDO-Me.** After six hours of stimulation the cells were lysed in 25 mM Tris-phosphate pH 7.8, 8 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100, and 7% glycerol during 15 min at RT in a horizontal shaker. Luciferase activity is measured using a microplate luminometer (Berthold) following the instructions of the luciferase assay kit (Promega, Madison, WI, USA). The RLUs are calculated and the EC50 and IRA (Intrinsic relative activity) values were determined relative to 150 μM deferoxamine (DFX) using the following equation: IRA coefficient = ($EC_{50\text{-}DFX}$ x E_{max}) / (EC_{50} x $E_{max\text{-}DFX}$), where EC_{50} and E_{max} denote EC_{50} and E_{max} of the agonist, and $EC_{50\text{-}DFX}$ and $E_{max\text{-}DFX}$ denote EC_{50} and E_{max} values of the standard agonist DFX (**Table II**).

None of the natural triterpenoids (OA; Olanolic acid, BA; Betulinic acid; UA, Ursolic acid, and MA; Maslimic acid) used as templates for the synthesis of the compounds included in the present invention were able to activate the EPO promoter as a surrogate marker of HIF-1α activation. In contrast all the triterpenoid derivatives of present invention clearly activated the HIF-1α pathway (**Table II**). Moreover, as shown in Table II below, compounds II to VI which are oleanolic acid derivatives of Formula I provide activation of the HIF pathway, while in contrast, an oleanolic derivative such as CDDO-Me, comprising a cyanide group at position 2 (corresponding to position B of Formula I) does not result in activation of the HIF pathway.

Thus, it can be concluded that the chemical modifications introduced in the compounds described are critical to inhibit the enzymatic activity of PHD2, and as a consequence, to activate the HIF pathway, but not for the binding of the compounds to the protein.

Table II also shows that GA and its hydroxamate derivative compound **XX,** do not activate the HIF-1α pathway. Thus, particularly, it can be concluded that the chemical modifications introduced at the position 28 of the backbone defined in the present invention is critical to inhibit the enzymatic activity of PHD2, and as a consequence, to activate the HIF pathway, but not for the binding of the compounds to the protein.

Compound	Efficacy HIF-1α (IRA coefficient)	Potency EC_{50} HIF-1α (μM)
OA		- (>50)
II	0,39	16.37
III	0,43	3.83
IV	0,15	11.39
V	0,16	6.71
VI	0,16	5.00
BA		- (>50)
VII	0,36	4.81
VIII	0,55	2.58
IX	0,34	2.41
X	0,48	3.24
XI	0,31	2.58
UA		- (>50)
XII	0,17	7.69
XIII	0,10	8.93
MA		-(>50)
XIV	0,10	7.10
XV	0,06	11.22
GA		-(>50)
XX		-(>50)
CDDO-Me		-(>50)

Table II. HIF-1α transactivation assays in NIH-3T3-EPO Luc fibroblast cells. NIH3T3-EPO-luc cell line stably transfected with the Epo-Luc plasmid, which contains three copies of the Hypoxia Response Element consensus sequence from the promoter of the erythropoietin gene fused to luciferase gene. The efficacy and potency for HIF-1α activation is shown.

Next, the activity of the compound in another cell type such as the keratinocyte cell line HaCaT-EPO-Luc was studied. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 in DMEM supplemented with 10% fetal calf serum (FBS), and 1% (v/v) penicillin/streptomycin. The cells ($1x10^5$/well in 24 well plates) were seeded the day before the assay and then stimulated with either DFX (150 μM) or with increasing concentrations of compounds **II** to **XV** for 6 h. Then, the cells were lysed in 25 mM Tris-phosphate pH 7.8, 8 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100, and 7% glycerol. Luciferase activity was measured in the cell lysate using a TriStar LB 941 multimode microplate reader (Berthold) following the instructions of the Luciferase Assay Kit (Promega, Madison, WI, USA). The above assay is illustrated by **Figure 2**, which shows the hypoximimetic effects of DFX and compounds **II** to **XV** in HaCaT-EPO-Luc cells. Data are given as percentage of activation considering DFX (150 μM) as 100% induction over untreated cells. A significant increase in luciferase activity was seen with all triterpenoid derivatives as compared with untreated cells.

To further study the target selectivity of the compounds described in the present invention the effect of the natural triterpenoids (OA; BA, UA, and MA), compounds **II** to **XV** of Formula (**I**) disclosed in present invention, and of comparative compound CDDO-Me on NF-κB inhibition, STAT-3 inhibition, Nrf2 activation and TGR5 activation was analyzed. For this study cell lines NIH-3T3-KBF-Luc, HeLa-STAT3-Luc, HaCaT-ARE-Luc and CHO-TGR5-CRE-luc were used respectively. The NIH3T3-KBF-Luc cell line stably transfected with the plasmid KBF-Luc plasmid, which contains three copies of NF-κB binding site (from major histocompatibility complex promoter), fused to a minimal simian virus 40 promoter driving the luciferase gene. Cells ($1x10^4$ /well) were seeded in 96-well plates, treated with increasing concentrations of the compounds **II** to **XV** for 15 min and then stimulated with 30 ng/ml TNFα. After 6 h the luciferase activity in the cell lysates was measured as indicated above. The RLU is calculated and the results are expressed as percentage of inhibition of NF-κB activity induced by TNFα (100% activation) (**Table III**).

Compound	IC_{50} NF-κB (μM)	IC_{50} STAT3 (μM)	EC_{50} NRF2 (μM)	IC_{50} NRF2 (μM)	EC_{50} TGR5 (μM)
OA	- (>50)	- (>50)	- (>50)	- (>50)	18.90
II	- (>50)	- (>50)	- (>50)	- (>50)	- (>50)
III	- (>50)	- (>50)	23.93	- (>50)	- (>50)
IV	- (>50)	- (>50)	10.04	- (>50)	- (>50)
V	- (>50)	- (>50)	- (>50)	- (>50)	- (>50)
VI	- (>50)	- (>50)	- (>50)	- (>50)	- (>50)
BA	- (>50)	- (>50)	9.02	- (>50)	22.15
VII	- (>50)	- (>50)	- (>50)	- (>50)	- (>50)
VIII	- (>50)	- (>50)	- (>50)	- (>50)	9.62
XI	- (>50)	- (>50)	- (>50)	- (>50)	17.50
X	- (>50)	- (>50)	- (>50)	- (>50)	- (>50)
XI	- (>50)	- (>50)	- (>50)	- (>50)	17.50
UA	- (>50)	- (>50)	38.36	- (>50)	11.46
XII	- (>50)	- (>50)	- (>50)	- (>50)	5.91
XIII	- (>50)	- (>50)	- (>50)	- (>50)	- (>50)
MA	- (>50)	- (>50)	- (>50)	- (>50)	10.19
XIV	- (>50)	- (>50)	- (>50)	- (>50)	- (>50)
XV	(>50)	(>50)	(>50)	(>50)	(>50)
CDDO-Me	1.20	2,38	40,94	0.06	(>50)

Table III. Effects on NF-κB, STAT-3, Nrf2 and TGR5 pathways.

Effect of compounds **II** to **XV,** OA; BA, UA, MA and of comparative compound CDDO-Me, on NF-κB inhibition (IC50), STAT-3 inhibition (IC50), Nrf2 activation (EC50) and inhibition (IC50) and TGR5 activation (EC50) we used the cell lines NIH-3T3-KBF-Luc, HeLa-STAT3-Luc, HaCaT-ARE-Luc and CHO-TGR5-CRE-luc respectively. The IC50 and EC50 data are shown.

The HeLa-STAT3-luc cells stably transfected with the plasmid 4xM67 pTATA TK-Luc. Cells (20 $x10^3$ cells/ml) were seeded in 96-well plates, treated with increasing concentrations of the compounds **II** to **XIV**, and of comparative compound CDDO-Me, for 15 min and then stimulated with IFN-γ 25 IU/ml. After 6 h the luciferase activity in the cell lysates was measured as indicated above. The RLU was calculated and the results expressed as percentage of inhibition of STAT3 activity induced by IFN-γ (100% activation) (**Table III**). The HaCaT-ARE-Luc cell line contains the Nqo1 Antioxidant Response Element (ARE)-Luc reporter plasmid. ARE is activated by all members of the CNC family of factors (Nrf1, Nrf2, Nrf3 and p45 NF-E2). The cells were cultivated in 96-well plates at the concentration of $25x10^3$ cells/well in a CO_2 incubator at 37°C. For induction of Nrf2 activation the cells were treated with increasing concentrations of the compounds **II** to **XV**, and of comparative compound CDDO-Me, for 6 h. As a positive control the cells were treated with 0.02 mM of the antioxidant Tert-butyl-hydroquinone (TBHQ). Luciferase activity in the cell lysates was measured as indicated above and the EC50 calculated (**Table III**). The CHO-TGR5-CRE-Luc cells stably transfected with the pTGR5 and CRE-Luc.

The CRE-responsive luciferase construct encodes the firefly luciferase reporter gene under the control of a minimal (m)CMV promoter and tandem repeats of the CRE transcriptional response element (TRE) and is useful to monitor cAMP signaling pathways activated by TGR5 agonists. Cells ($1x10^4$/well) were seeded in 96-well plates, treated with increasing concentrations of the compounds **II** to XV and CDDO-Me, for 6h. As a positive control the cells were treated with 10 μM of LCA (lithocholic acid). Luciferase activity in the cell lysates was measured as indicated above and the EC50 calculated (**Table III**).

None of the compounds inhibited the NF-κB and STAT3 pathways induced by TNFα and IFNγ respectively. In addition, none of the compounds activated the Nrf2 pathway and only compounds **VIII**, **XI** and **XII** showed agonistic TGR5 activity (**Table III**). In contrast, CDDO-Me clearly inhibited NF-κB and STAT-3 signaling pathways and activated the Nrf2 pathway. This result further demonstrated that the cyanide group is critical for some biological activities but is not required to activate the HIF-1α pathway (**Table III**).

Example 4. Triterpenoid derivatives stabilize HIF-1α and HIF-2α

To gain insight into the regulation of HIF-1α stabilization by the compounds described in the present invention the effect on HIF-1α expression in different cell types was investigated. Human oligodendrocyte MO13.3 cells were stimulated for 3 h with either 150 μDFX or 10 μM of Olenaolic acid (OA), compounds II, III, IV, V, VI (Fig. 3A), betulinic acid (BA), compounds VII, VIII, IX, X, XI (Fig. 3B), ursolic acid (UA), maslinic acid (MA), compounds XII, XIII, XIV and XV (Fig. 3C). After that, the cells were washed with PBS and incubated in 50 μl of NP-40 buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10% glycerol and 1% NP-40) supplemented with 10 mM NaF, 1 mM Na_3VO_4, 10 μg/ml leupeptine, 1 μg/ml pepstatin and aprotinin, and 1 μl/ml PMSF saturated. After centrifugation the supernatants were mixed with SDS sample buffer and boiled at 95ºC. Proteins were electrophoresed in 8-10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (20 V and 30 min per membrane). After blocking with non-fat milk or BSA in TBST buffer, primary antibodies were added. The washed membranes were incubated with appropriate secondary antibodies coupled to horseradish peroxidase that were detected by an enhanced chemiluminescence system (USB). The antibody against HIF-1α (610959) was from BD Biosciences and the antibody anti-β-actin (AC-74) was purchased from Sigma-Aldrich (Saint Louis, MO, USA).

All the compounds described in the present invention elevated HIF-1α protein level under normoxia conditions (21% O_2). The extent of induction was comparable to that of desferrioxamine (DFX), an iron chelator known to stabilize HIF-1α (**Fig. 3A, 3B and 3C**).

Next, Human Embryonic Kidney 293 cells (293T) were stimulated with the increasing concentrations of compound **VII** or with DFX (150 μM) during 3 h. After that, proteins isolation and western blots were performed as in **Fig. 3**. The antibody against HIF-1α (610959) was from BD Biosciences (USA), the antibodies anti-PHD1 (ab80361) and anti-PHD2 (ab109088) were from Abcam (Cambrigde, UK), and the antibody anti-β-actin (AC-74) was purchased from Sigma-Aldrich (Saint Louis, MO, USA).

The results clearly show that compound **VII**, as a representative of the compounds described in the present invention stabilized HIF-1α expression without affecting the expression of PDH1 and PDH2 (**Fig. 4**).

Since PDH2 and PDH3 also regulate the expression of HIF-2α, the effect of compound **VII** on HIF-2α stabilization in Human Islet-Derived Precursor Cells (hIPCs) obtained from Innoprot SL (Spain) (reference p10472) was investigated. hIPCs were stimulated with the increasing concentrations of compound **VII** or with DFX (150 μM) during 3 h. After that, proteins isolation and western blots were performed as in **Fig. 3**. The antibodies against HIF-2α (ab8365) and PHD3 (ab30782) were from Abcam (Cambrigde, UK), and the antibody anti-β-actin (AC-74) was purchased from Sigma-Aldrich (Saint Louis, MO, USA). (**Fig. 5**).
The results clearly show that compound **VII**, as a representative of the compounds described in the present invention stabilized HIF-2α expression without affecting the expression of PHD3 (**Fig. 5**).

Altogether, results indicate that compound **VII** binds PDH2 inhibiting its activity and as consequence HIF-1α and HIF-2α protein levels are stabilized.

Example 5. Compound VII induces angiogenesis.

To test the functional consequences of compound **VII** stimulation in a physiological model, endothelial cell tube formation was measured as a model of angiogenesis. CellPlayer™ GFP AngioKit-96 (Essen BioScience Inc., Welwyn Garden City, UK) was supplied as growing co-cultures of human matrix (normal human dermal fibroblast, NHDF) and endothelial cells (HUVEC) at the earliest stages of tubule formation. CellPlayer 96-well kinetic angiogenesis assay was performed according to the manufacturer's protocol. Briefly, lentivirally infected green

fluorescent protein (GFP)–HUVECs were cocultured with normal human dermal fibroblasts in a 96-well microplate. The plate was placed in IncuCyte, and images were automatically acquired in both phase and fluorescence every 6 hours for 7 days. At day 1, compound **VII** (1 and 2,5 μM) or VEGF (10 ng/ml) were added on the endothelial tube networks and kept throughout the experiment. Tube formation over the 7-day assay was quantified using the Essen BioScience Angiogenesis Analysis Module. This module provides multiple assay metrics, including tube length and branch point formation, which are used to assess angiogenic effects on network formation. Briefly, the fluorescent images were analyzed to generate a segmentation mask closely resembling the in vitro network. The mask was then refined to specifically identify tube-forming events, and the kinetic response was plotted using the IncuCyte and GraphPad Prism software (La Jolla, CA).

In **Figure 6** it is shown that compound **VII** 1μM as well as the positive controls (rFGF; 10 ng/ml and VEGFA; 10 ng/ml) increased significantly the network length in HUVEC cells.

Example 6. Compound VII increase the plasma levels of Erythropoietin (EPO).

Erythropoietin (EPO) is one of the earliest described and most sensitive HIF target genes; being positively regulated at the transcription level. Here, the ability of compound **VII** to regulated EPO levels in vivo was examined. Sixteen-week-old C57BL/6 male mice were treated intraperitoneally (i.p.) with Betulinic Acid (60 mg/kg) or compound **VII** (30 mg/kg or 60 mg/kg). Blood samples were taken under general anaesthesia 4 hours after treatment and the circulating levels of EPO in plasma were quantified using a mouse EPO ELISA kit (R&D Systems) according to manufacturer´s instructions. EPO values represent the mean ± SEM (n=3).

As shown in **Fig. 7** *in vivo* administration of compound **VII**, as a representative of the compounds described in the present invention, in mice (30 and 60mg/kg/day) strongly increased circulating EPO plasma levels. In contrast Betulinic acid (BA), the parental of compound **VII**, did not influence the levels of EPO in plasma.

Example 7. Effect of compound VII on 3-NP-induced cytotoxicity in striatal cells, and prevention of 3-NP induced Huntington's disease in mice.

7.1: Effect of compound VII on 3-NP-induced cytotoxicity in striatal cells

To investigate whether HIF activation in striatal Q7 and Q111 cells is sufficient to protect striatal neurons (provide neuroprotection), the effect of compound **VII** on 3-NP induced death was examined. In particular, the effect of 6 h pre-treatment with compound **VII** on 24 h of 3-

Nitropropionic acid (3-NP) exposure in ST*Hdh*$^{Q7/Q7}$ (striatal Q7) and ST*Hdh*$^{Q111/Q111}$ (striatal Q111) cells was studied.

ST*Hdh*$^{Q111/Q111}$ cells express a mutated form of the huntingtin protein and ST*Hdh*$^{Q7/Q7}$ cells express the wild type form of this protein. Clonal striatal cell lines established from E14 striatal primordia of *Hdh*$^{Q111/Q111}$ (mutant) and *Hdh*$^{Q7/Q7}$ (wild-type) knock-in mouse littermates were immortalized using a replication defective retrovirus transducing the tsA58/U19 large T-antigen (Trettel F. *et al.*, Hum Mol Genet. 2000; 9:2799–2809). Striatal Q7 and Q111 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 25 mM D-glucose, 1 mM L-glutamine, 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, and 400 µg/mL Geneticin and were incubated at 33°C with 5% CO_2.

3-Nitropropionic acid (3-NP) is a potent irreversible inhibitor of mitochondrial complex II enzyme and leads to mitochondrial dysfunction and oxidative stress.

ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ cells (10^4 cells/well in 96-well plates) were incubated with YOYO-1 (Life Technologies) and then treated with 3-NP (10 mM) and or DFX (50 µM) as a positive control for PHDs inhibition. YOYO-1 is diluted in cell culture medium and added to a final concentration of 0.1 µM to both experimental and control wells. YOYO-1 is a cell impermeant cyanine dimer nucleic acid stain that can only enter cells with a compromised plasma membrane and fluorescently stain the nuclear DNA. The uptake of YOYO-1 by damaged cells correlates with the increase in YOYO-1 fluorescence. Treated cells are placed in an Incucyte FLR imaging system, and the YOYO-1 fluorescence is measured after 24 h of 3-NP treatment. Following the incubation period object counting analysis was performed using the Incucyte FLR software to calculate the total number of YOYO-1 fluorescence positive cells and total DNA containing objects (end point). The cytotoxicity index is calculated by dividing the number of YOYO-1 fluorescence positive objects by the total number of DNA containing objects for each treatment group and converted to percentage of cell death.

ST*Hdh*$^{Q111/Q111}$ cells were more sensitive than ST*Hdh*$^{Q7/Q7}$ cells to the exposure to 3-NP and compound **VII** clearly provided a significant level of neuroprotection to 3-NP-induced cytotoxicity (**Fig. 8**). Although to a lesser extent, DFX also protected the cells from the cytotoxic activity of 3-NP.

<u>**7.2**: Prevention of 3-NP induced Huntington's disease in mice</u>

The intoxication of mice with 3-Nitropropionic acid (3-NP), results in a myriad of neurological, biochemical and histological effects that are reminiscent of some aspects of Huntington disease

(HD) pathology. 3NP-treated mice exhibited high scores in hindlimb clasping, dystonia, kyphosis and in general locomotor activity compared to control animals (non-intoxicated with 3-NP).

Striatal neurodegeneration was induced in 16-week-old C57BL/6 male mice (Harlan Ibérica, Barcelona, Spain) by six intraperitoneal (i.p.) injections of 3-nitropropionic acid (3NP) (30 mg/kg; one injection each every 12 h prepared in PBS). 3NP-treated animals and the non-lesioned control group (injected with PBS) were used for pharmacological studies with Betulinic acid (30 mg/Kg) and compound **VII** (30 mg/Kg). Treatments consisted of 5 i.p. injections every 24h with 50 mg/kg the test compounds or vehicle (10 % DMSO plus 6,2 % Tween 20 in saline buffer), with the first injection 24 h prior the first 3NP injection and the rest of doses 30 min before the injections of 3NP. Mice were subjected to behavioral tests for determining their neurological status. General locomotor activity, the hindlimb clasping and dystonia, and the truncal dystonia were evaluated. All behavioral tests were conducted prior to drug injections to avoid acute effects of the compounds under investigation. All behavioral tests were conducted prior to drug injections to avoid acute effects of the compounds under investigation and all animals were euthanized 12 h after the last injection of 3NP. Once euthanized, animals were dissected and their brains were rapidly removed. The right hemisphere was used to dissect the striatum, which was quickly frozen in RNAlater (Sigma-Aldrich, Germany) to analyzed inflammatory markers were by Real Time PCR. The left hemisphere was fixed in fresh 4% paraformaldehyde (in 0.1M phosphate buffered-saline) for 48 hours at 4ºC and embedded in paraffin wax for histological analysis.

Figure 9 shows that **compound VII** clearly alleviated the clinical symptoms induced by 3-NP intoxication. Betulinic acid (BA) also showed some neuroprotective activity although to a lesser extent.

Next, striatal parenchyma of 3NP-lesioned mice was also used for analysis of some histological and molecular markers related to inflammation and neurodegeneration, which are affected in this experimental model. The striatal parenchyma of these 3NP-treated animals showed an important reduction in Nissl-stained cells, which indicates an important degree of neuronal death caused by 3NP, that was clearly prevented by treatment with compound **VII** but not by Betulinic acid (BA) (**Figure 10**).

In addition, compound **VII**-mediated neuroprotection was associated with reduced 3NP-induced microgliosis and astrogliosis as determined by Iba1 and GFAP immunohistochemistry (**Figure**

11). For this test, intoxicated mice with 3-Nitropropionic acid (3-NP) were used for determining microglia activation and astrogliosis including a Control with non-intoxicated mice (3NP, 3NP+BA and 3NP + Compound **VII**). Iba-1 and glial fibrillary acidic protein (GFAP) expression were determined by immunostaining of brain sections through the different group of mice. Quantification of the different markers was performed with Image J software. Total average number of microglia ($Iba1^{+}$) and astrocytes ($GFAP^{+}$) is shown in **Figure 11**.

Finally, the expression of inflammatory enzymes COX-2 and iNOs was significantly up regulated in 3NP-lesioned mice in parallel to increased expression of proinflammatory cytokines IL-1β and IL-6. The mRNA expression for COX-2 (**Figure 12D**), IL-1β (**Figure 12B**), IL-6 (**Figure 12A**) and iNOS(**Figure 12C**), was down regulated in 3NP+compound **VII** (30 mg/kg) treated mice compared with 3NP+Vehicle mice. Betulinic acid (BA) treatment (30 mg/kg) also inhibited the expression of inflammatory markers (as shown in **Fig. 12**). Expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Values are expressed as means ± SEM for 6 animals *per* group

Example 8. Real-Time Quantitative PCR used in the invention (example 7).

Total RNA was isolated from striata (3NP model) using RNeasy Lipid Tissue Mini Kit (Qiagen, GmbH). The total amount of RNA extracted was quantitated by spectrometry at 260 nm and its purity from the ratio between the absorbance values at 260 and 280 nm. Genomic DNA was removed to eliminate DNA contamination. Single-stranded complementary DNA was synthesized from up to 1 μg of total RNA (pool from at least 3 animals per group) using iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). The reaction mixture was kept frozen at -20ºC until enzymatic amplification. The iQ™ SYBR Green Supermix (Bio-Rad) was used to quantify mRNA levels for COX-2, IL-6, IL-1β, and iNOS. Real-time PCR was performed using a CFX96 Real-Time PCR Detection System (Bio-Rad). The GAPDH housekeeping gene was used to standardize the mRNA expression levels in every sample. Expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Sequences of oligonucleotide primers are given in **Table IV**.

Table IV. List of mouse primer sequences used in quantitative Polymerase Chain Reaction.

Gene	Forward	Reverse
IL-6	5′-GAACAACGATGATGCACTTGC-3′	5′-TCCAGGTAGCTATGGTACTCC-3
iNOS	5′-AACGGAGAACGTTGGATTTG-3′	5′-CAGCACAAGGGGTTTTCTTC-3′
COX-2	5′-TGAGCAACTATTCCAAACCAGC-3′	5′-GCACGTAGTCTTCGATCACTATC-3′
IL-1β	5′-CTCCACCTCAATGGACAGAA-3′	5′-GCCGTCTTTCATTACACAGG-3′
GAPDH	5′-TGGCAAAGTGGAGATTGTTGCC-3′	5′-AAGATGGTGATGGGCTTCCCG-3′

The present results substantiate the therapeutic use of the compounds described in the present inventions, for the clinical management of conditions and diseases the treatment of which is responsive to HIF activation such as stroke, cerebral palsy, traumatic injuries, neurodegenerative diseases such as Multiple Sclerosis, Huntington disease, Alzheimer disease and Parkinson disease, IBD, myocardial ischaemia–reperfusion injury, acute lung injury, organ transplantation, acute kidney injury and arterial diseases.

Example 9. Effect of compound VII on a high fat diet (HFD) model of diabetes and metabolic pathologies.

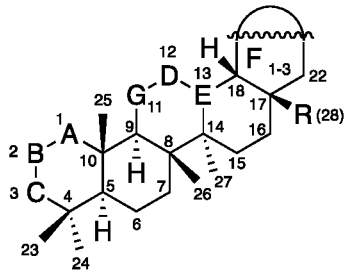
High-fat diet–fed mouse is a widely used model for impaired glucose tolerance (IGT) and type 2 diabetes. Eight-week-old male C57BL/6 mice (Charles River-France) were used for experiments. Mice were maintained under controlled conditions [12 h light/dark cycle; temperature 20ºC (± 2ºC) and 40–50% relative humidity] with free access to tap water and standard rodent chow ad libitum. After 1 week of acclimatization, mice were divided into two groups and fed ad libitum with a standard diet -CD- (Code U8220G10R, SAFE Diets, Augy, France) or a High-Fat diet -HFD- (D12451; Research Diets, New Brunswick, NJ) in order to induce obesity for 15 weeks (diet-induced obesity, DIO). In order to assess the potential beneficial metabolic effects of compound **VII** in this model of DIO, pharmacological administration of the compound (30 mg/kg body weight) was performed by intraperitoneal (i.p.) injection every 24 h from 12th to 15th week of diet exposure. Control animals received the corresponding vehicle injections (1:1:18 Ethanol:Cremophor:Saline).

To ensure the effectiveness of HFD feeding regimen and assess the efficacy of compounds in terms of amelioration of the metabolic phenotype, body composition analyses of fat and lean masses were performed by QMR (quantitative magnetic resonance) using the EchoMRI™ 700 analyzer (Houston, TX, software v.2.0). In this sense, MRI scans were taken in three time points during the experimental period: before starting diet exposure, after 12 week of feeding regimen (coinciding with start of treatment) and at the end of experimental procedures (on the 15th week of diet exposure). Additionally, five days before sacrifice, glucose tolerance test (GTT) was performed in overnight food-deprived mice. To this end, mice were injected intraperitoneally with 2 mg of glucose/g body weight and blood glucose was measured before and 30, 60 and 120 min after injection utilizing an Accu-Chek Advantage® (Roche) glucometer. At the end of the experiment, mice were sacrificed and blood samples were collected using EDTA-coated tubes and plasma stored at −80 °C. Plasma triglycerides were measured using and enzymatic colorimetric kit (QCA, Barcelona, Spain).

We found that compound **VII** alleviated weight gain (**Fig. 13A**), increase in Fat Mass (**Fig.13B**) and adiposity (**Fig. 13C**). Moreover, compound **VII** improved glucose tolerance (**Fig. 14**) and normalize the plasma levels of triglycerides (**Fig. 15**) in HFD mice. Our results are in agreement with previous report showing that PDH inhibitors improves glucose and lipid metabolism in murine models of obesity and diabetes induced by HFD (Rahtu-Korpela et al., Diabetes. 2014 Oct;63(10):3324-33). Therefore, said results indicate that the compounds of the invention are useful in the treatment of diabetes or other related metabolic pathologies such as hypertriglyceridemia.

CLAIMS

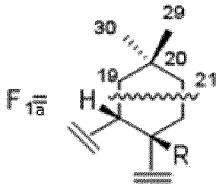
1. A triterpene derivative of Formula (**Ia**) or stereoisomers, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof,

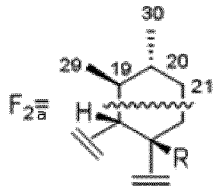


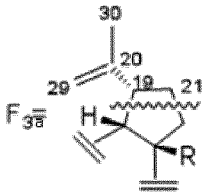
Formula (**Ia**)

wherein independently,

- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;
- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];
- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen; and wherein said heterocyclic ring is a five-membered ring comprising one nitrogen and one oxygen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from F_{1a}, F_{2a} or F_{3a};







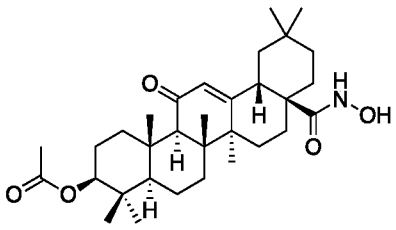
– G is selected from a methylene (-CH_2-) or a carbonyl [-C(=O)-]; and

– R is a hydroxamate group (-CONHOH);

and wherein,

- when C is an acyloxymethine [-CH(OCOR')-], the triterpene derivative of Formula (**Ia**) is

(V)



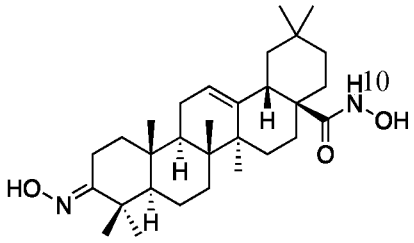
(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide;

- when B is a methylene (-CH_2-), C is a hydroxymethine [-CH(OH)-], D-E is a double carbon-carbon bond, G is a methylene (-CH_2-) and R is a hydroxamate (-CONHOH), F is F_{3a};
- when B is a methylene (-CH_2-), C is a hydroxymethine [-CH(OH)-], D-E is a single carbon-carbon bond, G is a methylene (-CH_2-) and R is a hydroxamate (-CONHOH), F is selected from F_{1a} or F_{2a};

- when B is a methylene (-CH_2-), C is an oxime [-C(=N-OH)-], D-E is a double carbon-carbon bond, G is a methylene (-CH_2-) and R is a hydroxamate (-CONHOH), F is selected from F_{1a} or F_{3a}.

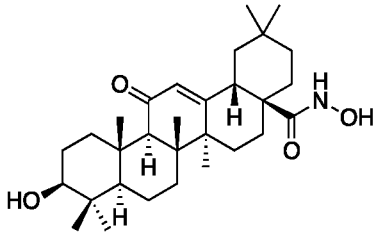
2. A triterpene derivatives according to claim 1, wherein said triterpene derivative is selected from:

(III)



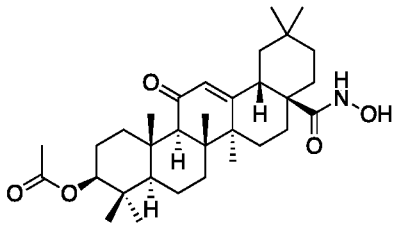
3-Hydroxyimino-*N*-hydroxy-olean-12-en-28-amide

(IV)



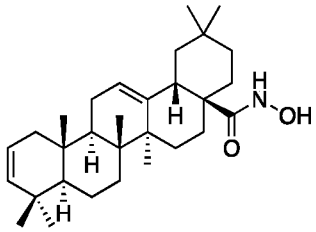
(3β) 3-Hydroxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(V)



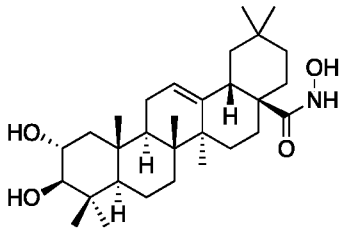
(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(VI)



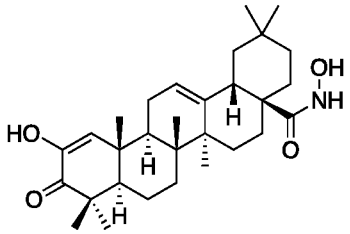
Oleana-2,12-dien-*N*-hydroxy-28-amide

(XIV)



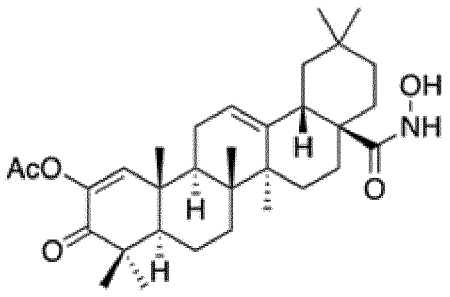
(2α,3β) 2,3-Dihydroxy-*N*-hydroxy-olean-12-en-28-amide

(XV)

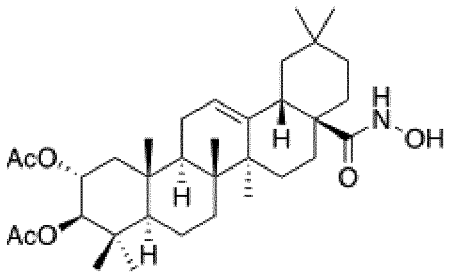


2-Hydroxy-3-oxo-oleana-1,12-dien-*N*-hydroxy-28-amide

3. Intermediate compounds in the synthesis of compounds of any of claims 1 or 2 selected from **XVIII** or **XIX**:



(XVIII)



(XIX)

4. A triterpene derivative of Formula (**Ia**), according to any of claims 1 or 2 for use as medicament.

5. A triterpene derivative of Formula (**Ia**), according to any of claims 1 or 2 for use in the treatment of a condition or disease responsive to the activation of the HIF pathway.

6. A triterpene derivative of Formula (**Ia**) for use, according to claim 5, wherein the condition or disease responsive to the activation of the HIF pathway is selected from stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases.

7. A triterpene derivative of Formula (**Ia**) for use, according to claim 5, wherein the condition or disease responsive to the activation of the HIF pathway is selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases.

8. A pharmaceutical composition comprising at least one triterpene derivative of Formula (**Ia**) according to any of claims 1-2 as a first active ingredient and at least one excipient or carrier, and optionally further comprising at least a second active ingredient.

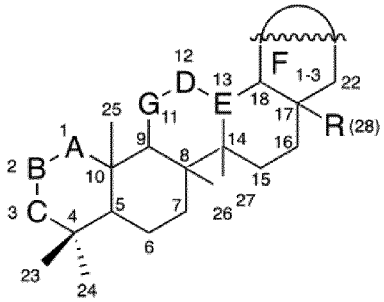
9. A pharmaceutical composition according to claim 8 for use as medicament.

10. A pharmaceutical composition according to claim 8 for use in the treatment of a condition or disease responsive to the activation of the HIF pathway.

11. A pharmaceutical composition for use, according to claim 10, wherein the condition or disease responsive to the activation of the HIF pathway is selected from stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases.

12. A pharmaceutical composition for use, according to claim 10, wherein the condition or disease responsive to the activation of the HIF pathway is selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases.

13. A triterpene derivative of Formula (**I**) or, stereoisomers, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof,

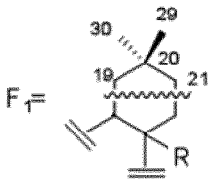


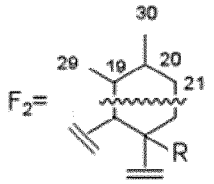
Formula (**I**)

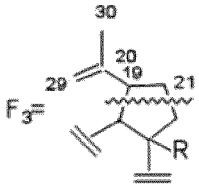
wherein independently,

- A-B is selected from a single carbon-carbon bond or a double carbon-carbon bond;
- B is selected from a methylene (-CH_2-), an olefin methine (=CH-), a hydroxymethine [-CH(OH)-], or a hydroxylated olefin carbon [-C(OH)=];

- B-C is selected from a single carbon-carbon bond or a double carbon-carbon bond; or be part of a heterocyclic ring comprising one or more heteroatoms wherein at least one of said heteroatoms is nitrogen; and wherein said heterocyclic ring is a five membered ring comprising one nitrogen and one oxygen;
- C is selected from a hydroxymethine [-CH(OH)-], an acyloxymethine [-CH(OCOR')-], an olefin methine (=CH-), a carbonyl [-C(=O)-], an oxime [-C(=N-OH)-] or an hydrazone [-C(=N-NH_2)-], wherein R' is methyl;
- D-E is a single or a double carbon-carbon bond;
- F is selected from F_1, F_2 or F_3;





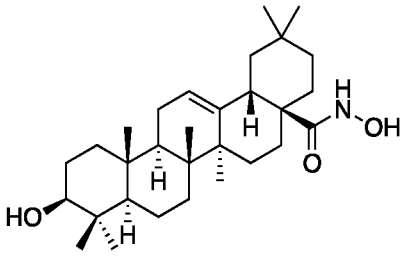


- G is selected from a methylene (-CH_2-) or a carbonyl [-C(=O)-]; and
- R is a hydroxamate group (-CONHOH);

for use in the treatment of a condition or disease responsive to the activation of the HIF pathway.

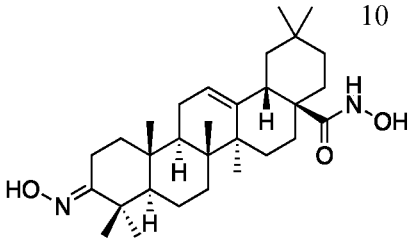
14. A triterpene derivative for use, according to claim 13, wherein said triterpene derivative is selected from (**II**), (**III**), (**IV**), (**V**), (**VI**), (**VII**), (**VIII**), (**X**), (**XI**), (**XII**), (**XIII**), (**XIV**) or (**XV**).

(II)



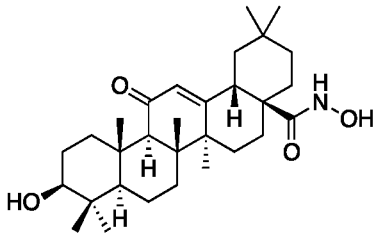
(3β) 3-Hydroxy-*N*-hydroxy-olean-12-en-28-amide

(III)



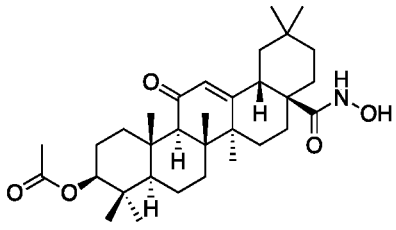
3-Hydroxyimino-*N*-hydroxy-olean-12-en-28-amide

(IV)



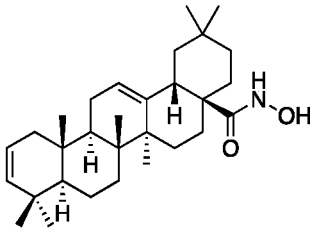
(3β) 3-Hydroxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(V)



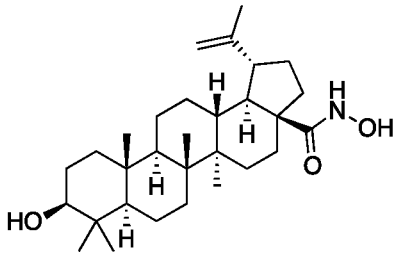
(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(VI)



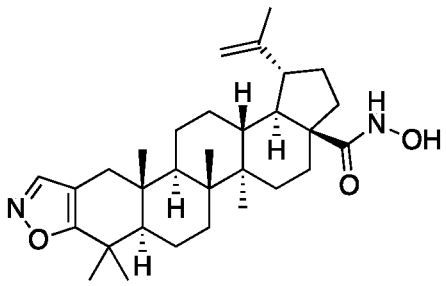
Oleana-2,12-dien-*N*-hydroxy-28-amide

(VII)



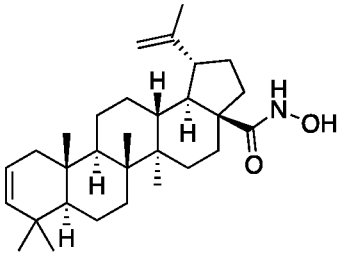
(3β) 3-Hydroxy-*N*-hydroxy-lup-20(29)-en-28-amide

(VIII)



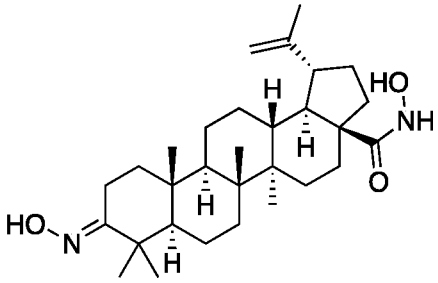
Lup-2-eno[2,3-d]isoxazol-*N*-hydroxy-28-amide

(X)



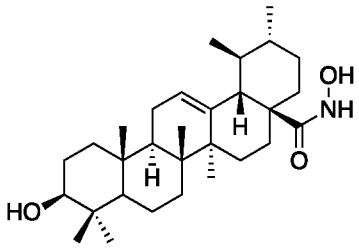
Lupa-2,20(29)-dien-*N*-hydroxy-28-amide

(XI)



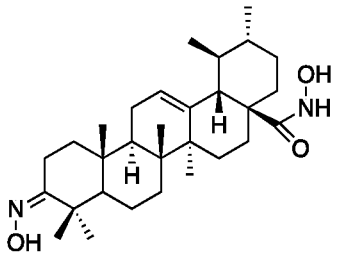
3-Hydroxyimino-*N*-hydroxy-lup-20(29)-en-28-amide

(XII)



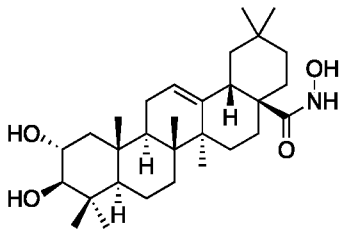
(3β) 3-Hydroxy-*N*-hydroxy-urs-12-en-28-amide

(XIII)



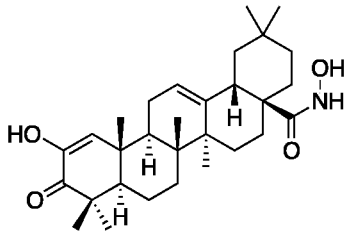
3 Hydroxyimino-*N*-hydroxy-urs-12-en-28-amide

(XIV)



(2α,3β) 2,3-Dihydroxy-*N*-hydroxy-olean-12-en-28-amide

(XV)



2-Hydroxy-3-oxo-oleana-1,12-dien-*N*-hydroxy-28-amide

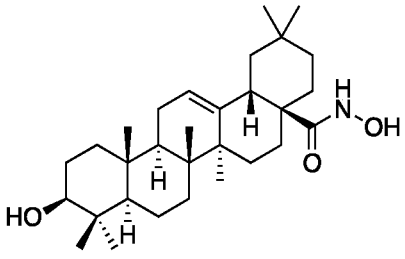
15. A triterpene derivative for use, according to any of claims 13 or 14, wherein said condition or disease responsive to the activation of the HIF pathway is selected from stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases.

16. A triterpene derivative for use, according to any of claims 13 or 14, wherein said condition or disease responsive to the activation of the HIF pathway is selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases.

17. A pharmaceutical composition comprising at least one triterpene derivative of Formula (**I**), or stereoisomers, pharmaceutically acceptable salts or pharmaceutically acceptable solvates thereof, according to claim 13; and at least one excipient or carrier; for use in the treatment of a condition or disease responsive to the activation of the HIF pathway.

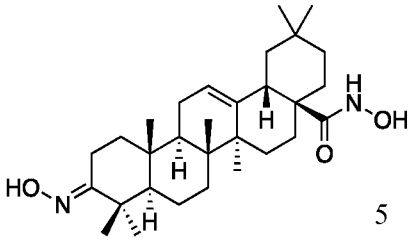
18. A pharmaceutical composition for use, according to claim 17, wherein said at least one triterpene derivative is selected from:

(II)



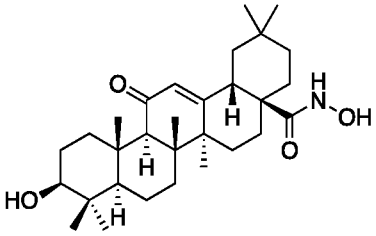
(3β) 3-Hydroxy-*N*-hydroxy-olean-12-en-28-amide

(III)



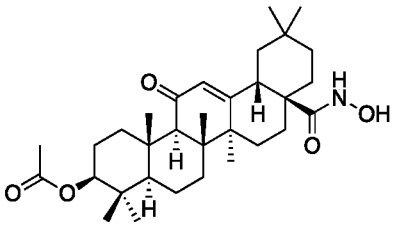
3-Hydroxyimino-*N*-hydroxy-olean-12-en-28-amide

(IV)



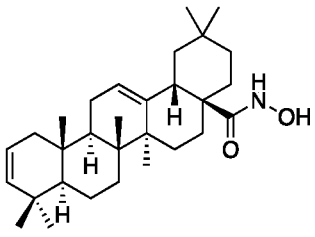
(3β) 3-Hydroxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(V)



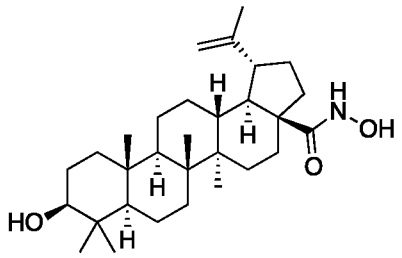
(3β) 3-Acetyloxy-11-oxo-olean-12-en-*N*-hydroxy-28-amide

(VI)



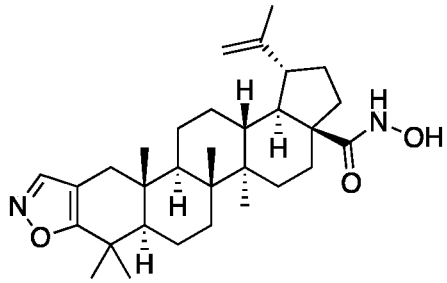
Oleana-2,12-dien-*N*-hydroxy-28-amide

(VII)



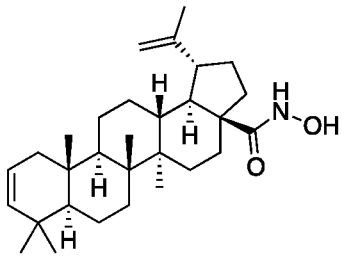
(3β) 3-Hydroxy-*N*-hydroxy-lup-20(29)-en-28-amide

(VIII)



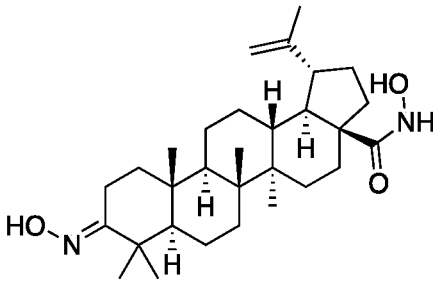
Lup-2-eno[2,3-d]isoxazol-*N*-hydroxy-28-amide

(X)



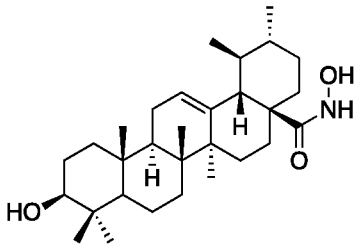
Lupa-2,20(29)-dien-*N*-hydroxy-28-amide

(XI)



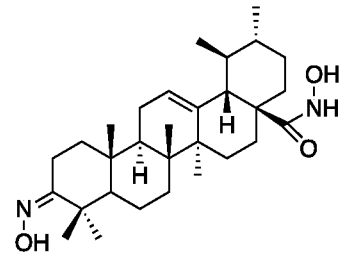
3-Hydroxyimino-*N*-hydroxy-lup-20(29)-en-28-amide

(XII)



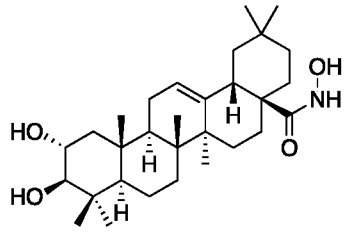
(3β) 3-Hydroxy-*N*-hydroxy-urs-12-en-28-amide

(XIII)



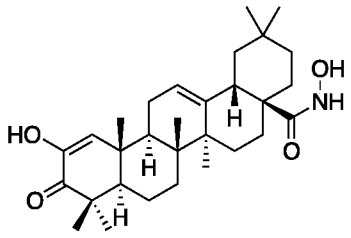
3 Hydroxyimino-*N*-hydroxy-urs-12-en-28-amide

(XIV)



(2α,3β) 2,3-Dihydroxy-*N*-hydroxy-olean-12-en-28-amide

(XV)



2-Hydroxy-3-oxo-oleana-1,12-dien-*N*-hydroxy-28-amide

19. A pharmaceutical composition for use, according to any of claims 17 or 18, wherein said condition or disease responsive to the activation of the HIF pathway is selected from stroke, cerebral palsy, traumatic injuries and neurodegenerative diseases

20. The pharmaceutical composition for use, according to any of claims 17 or 18, wherein said condition or disease responsive to the activation of the HIF pathway is selected from IBD, myocardial ischaemia–reperfusion injury, acute lung injury, diabetic and chronic wounds, organ transplantation, acute kidney injury or arterial diseases.

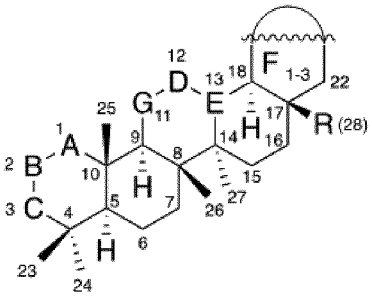
21. A triterpene derivative of Formula (**Ia**) for use, according to claim 5, wherein the condition or disease responsive to the activation of the HIF pathway is independently selected from diabetes, hyperlipidemia or hypertriglyceridemia.

22. A pharmaceutical composition for use, according to claim 10, wherein the condition or disease responsive to the activation of the HIF pathway is independently selected from diabetes, hyperlipidemia or hypertriglyceridemia.

23. A triterpene derivative of Formula (**I**) for use, according to any of claims 13 or 14, wherein the condition or disease responsive to the activation of the HIF pathway is independently selected from diabetes, hyperlipidemia or hypertriglyceridemia.

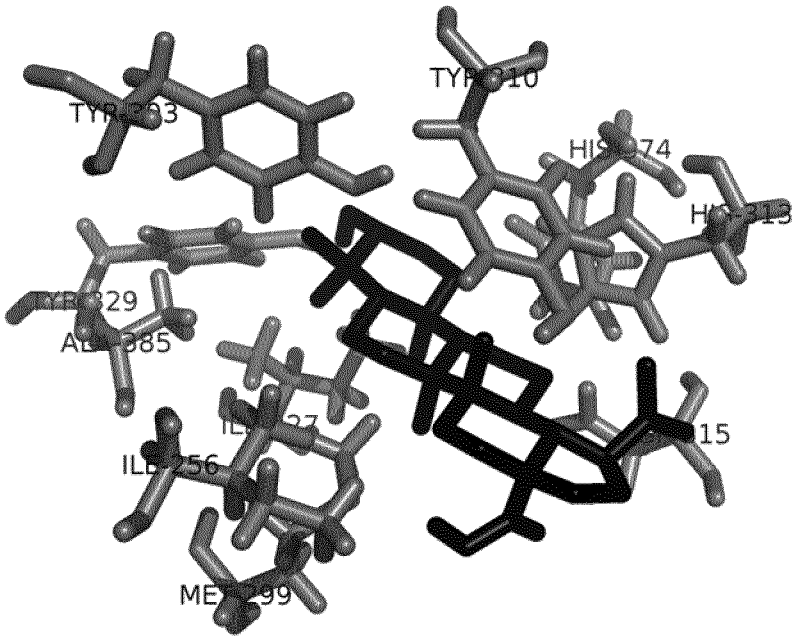
24. A pharmaceutical composition for use, according to any of claims 17 or 18, wherein the condition or disease responsive to the activation of the HIF pathway is independently selected from diabetes, hyperlipidemia or hypertriglyceridemia.

25. A triterpene derivative of Formula I for use, according to claim 13, wherein said triterpene derivative is a triterpene derivative of Formula (Ib):

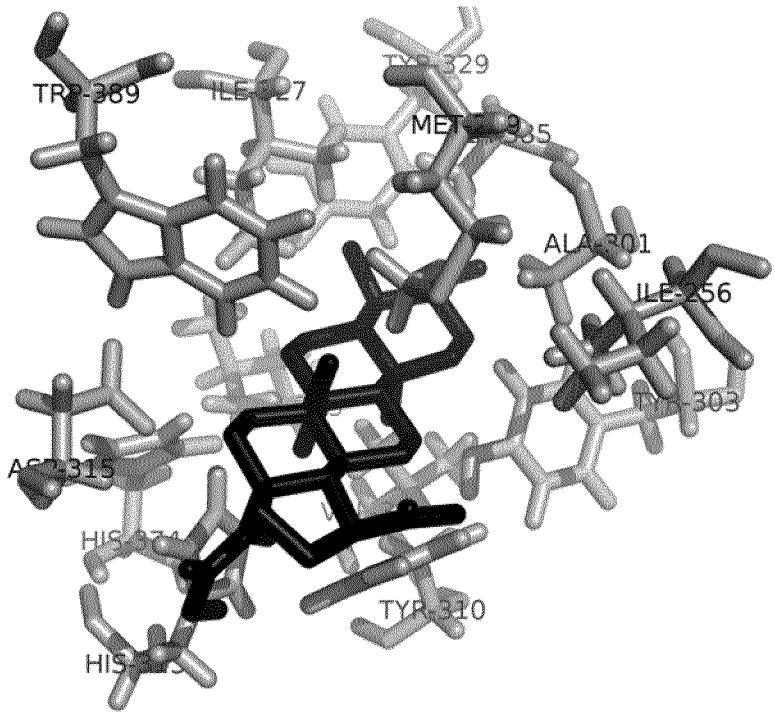

12
D
13
18
F
1-3
22
25
G
11
E
17
R (28)
1
A
9
14
H
16
2
B
10
8
15
H
3
C
4
5
7
27
26
H
6
23
24

Formula (Ib)

FIGURE 1



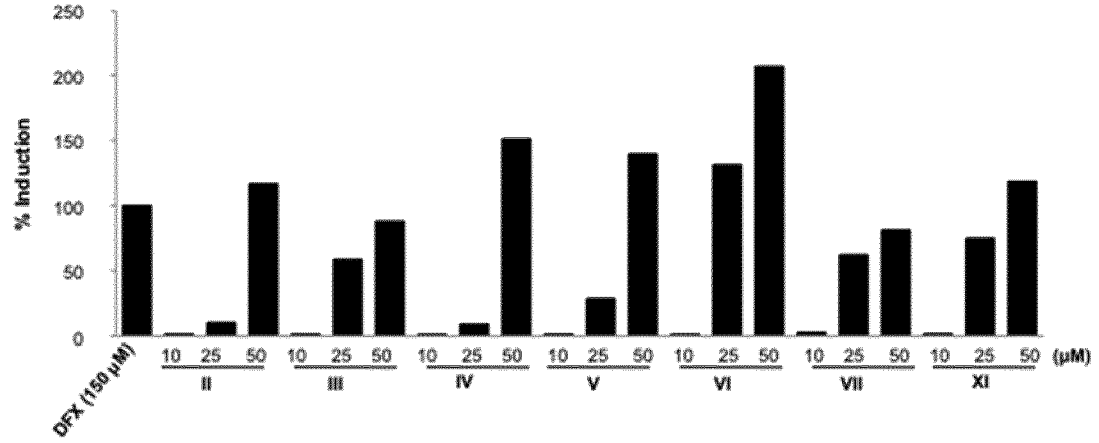
1A



1B

FIGURE 2

2A



2B

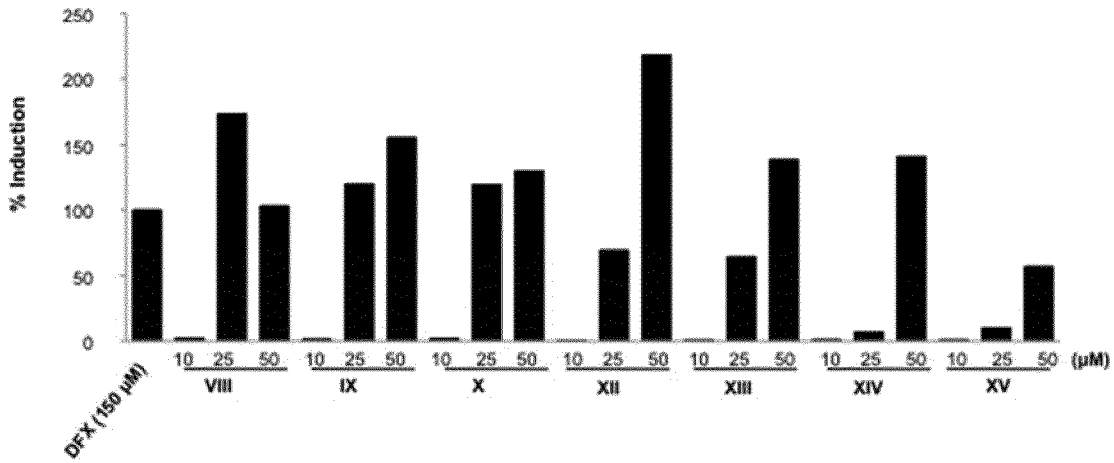


FIGURE 3

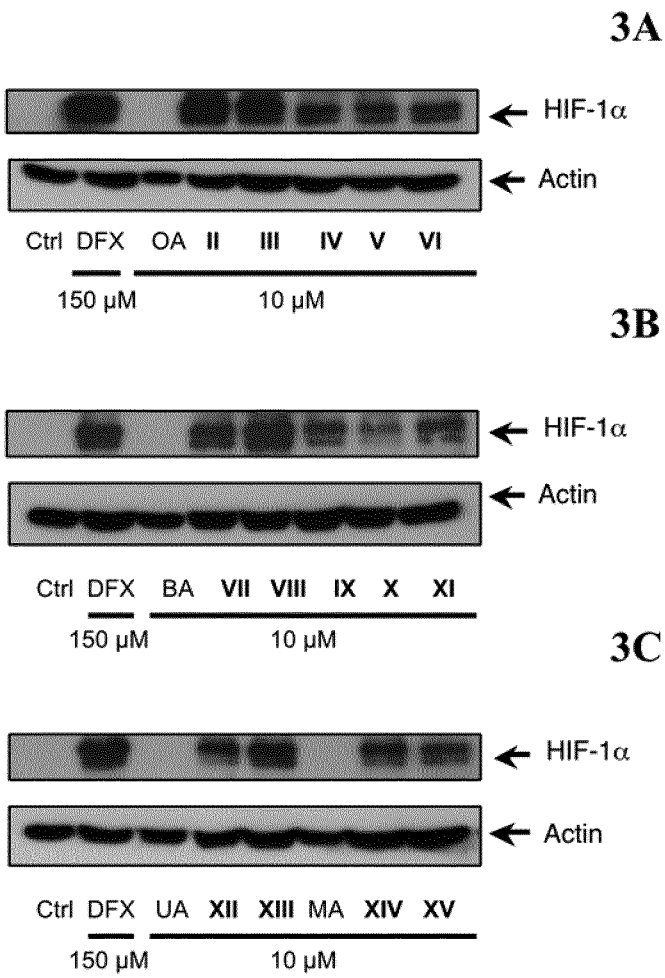

3A
HIF-1α
Actin
Ctrl DFX OA II III IV V VI
150 μM
10 μM
3B
HIF-1α
Actin
Ctrl DFX BA VII VIII IX X XI
150 μM
10 μM
3C
HIF-1α
Actin
Ctrl DFX UA XII XIII MA XIV XV
150 μM
10 μM

FIGURE 4

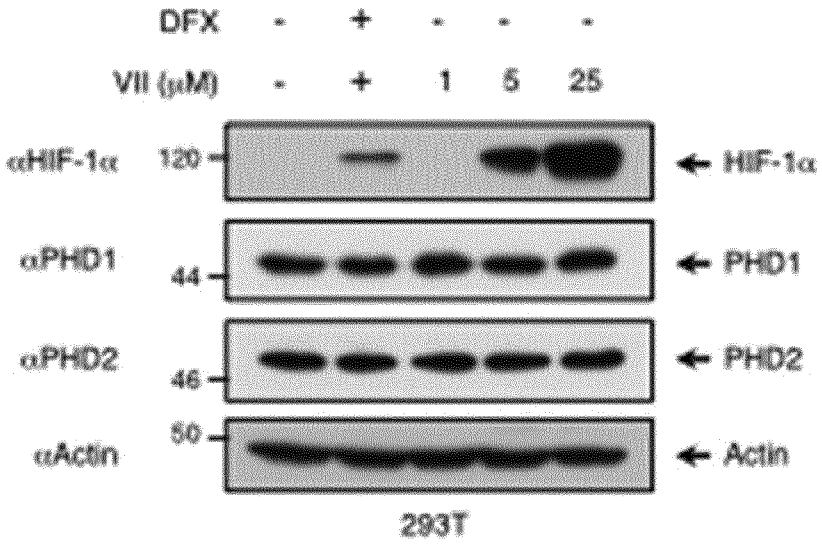

DFX - + - - -
VII (μM) - + 1 5 25
αHIF-1α
120
HIF-1α
αPHD1
44
PHD1
αPHD2
46
PHD2
αActin
50
Actin
293T

FIGURE 5

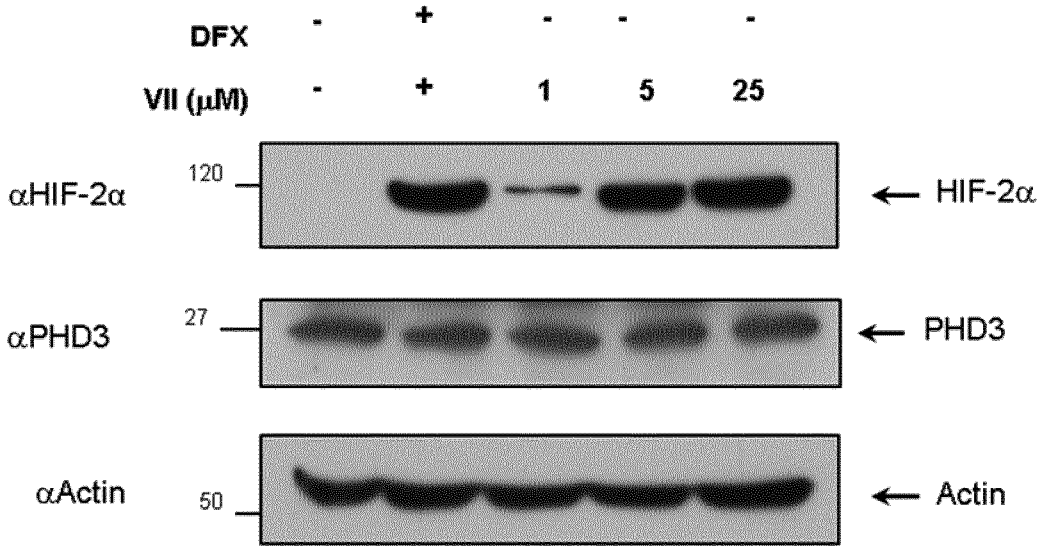

DFX
-
+
-
-
-
VII (μM)
-
+
1
5
25
αHIF-2α
120
HIF-2α
αPHD3
27
PHD3
αActin
50
Actin

FIGURE 6

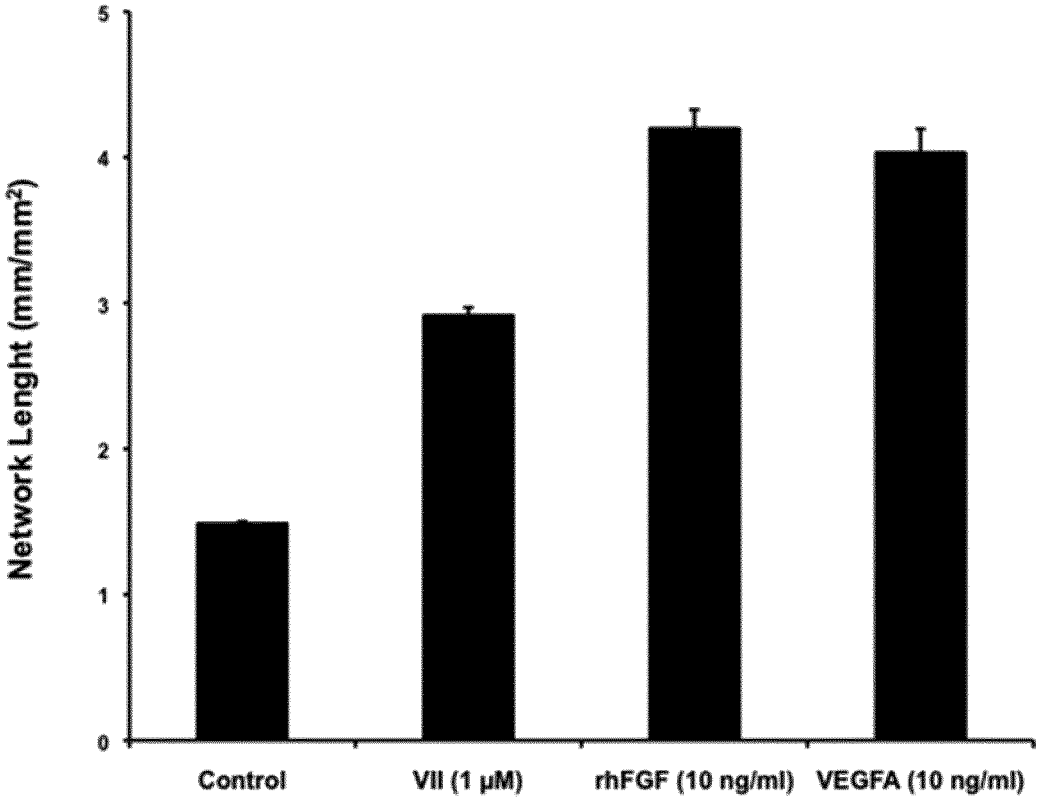

Network Lenght (mm/mm²)
0
1
2
3
4
5
Control
VII (1 µM)
rhFGF (10 ng/ml)
VEGFA (10 ng/ml)

FIGURE 7

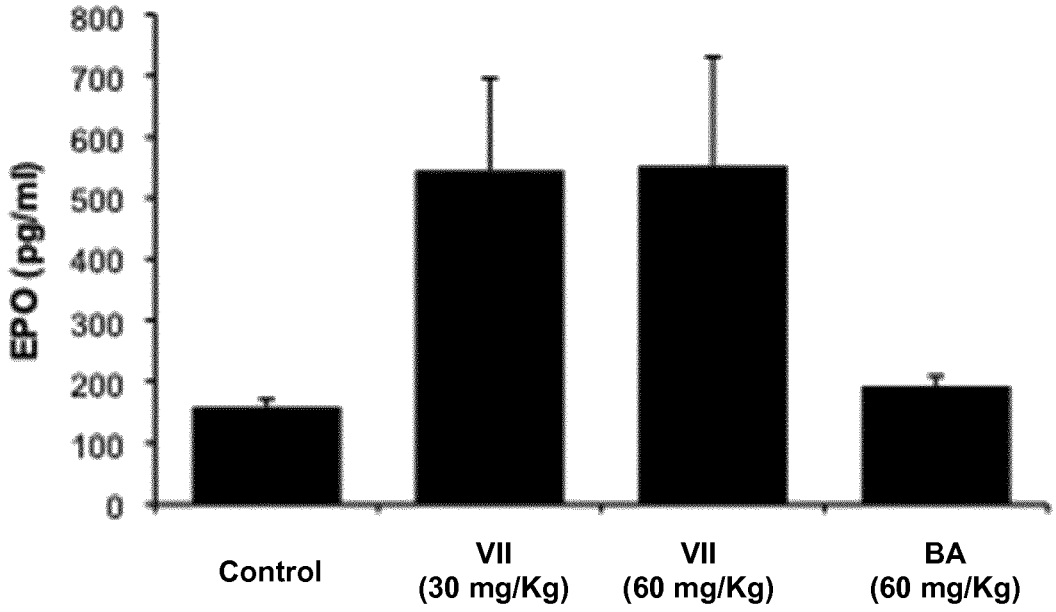

800
700
600
500
400
300
200
100
0
EPO (pg/ml)
Control
VII
(30 mg/Kg)
VII
(60 mg/Kg)
BA
(60 mg/Kg)

FIGURE 8

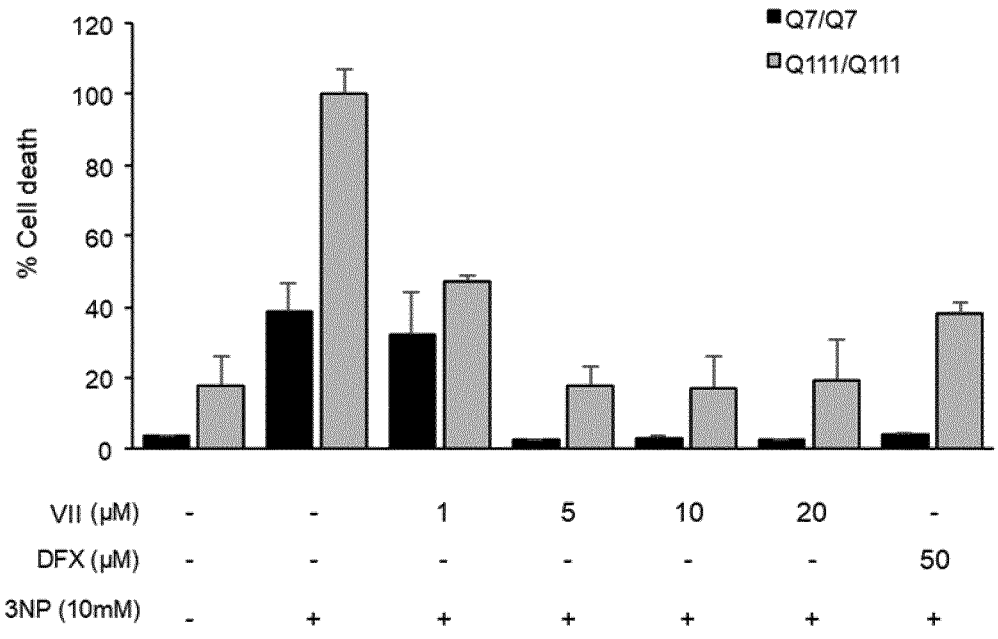

Q7/Q7
Q111/Q111
% Cell death
120
100
80
60
40
20
0
VII (µM) - - 1 5 10 20 -
DFX (µM) - - - - - - 50
3NP (10mM) - + + + + + +

FIGURE 9

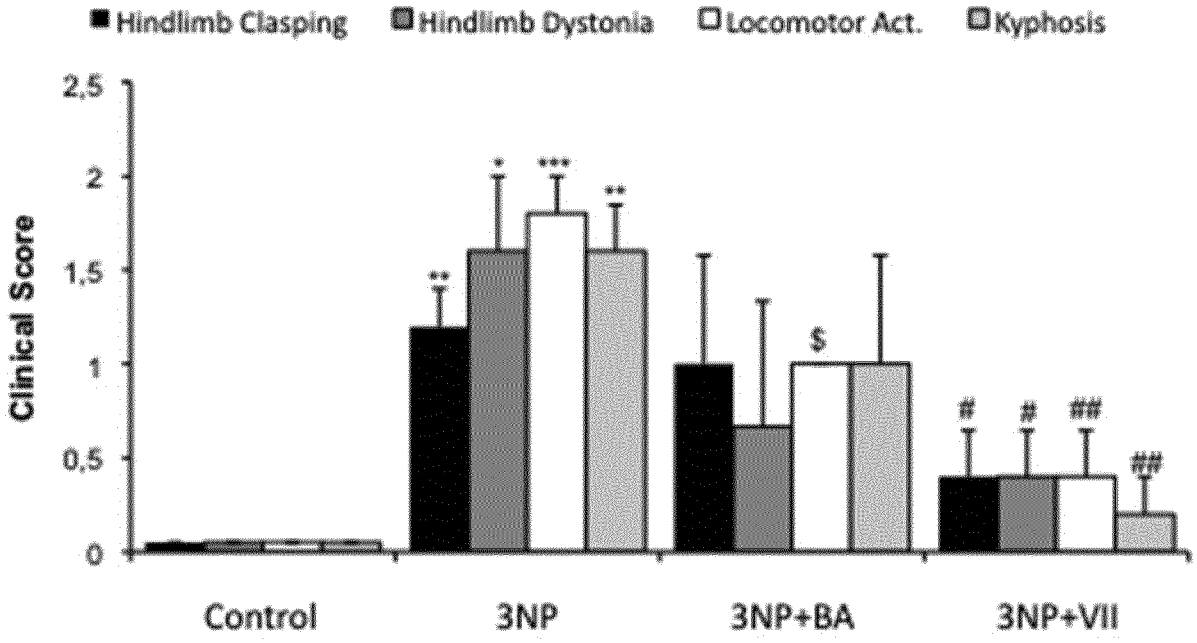

Hindlimb Clasping
Hindlimb Dystonia
Locomotor Act.
Kyphosis
Clinical Score
2,5
2
1,5
1
0,5
0
**
*
***
**
$
#
#
##
##
Control
3NP
3NP+BA
3NP+VII

FIGURE 10

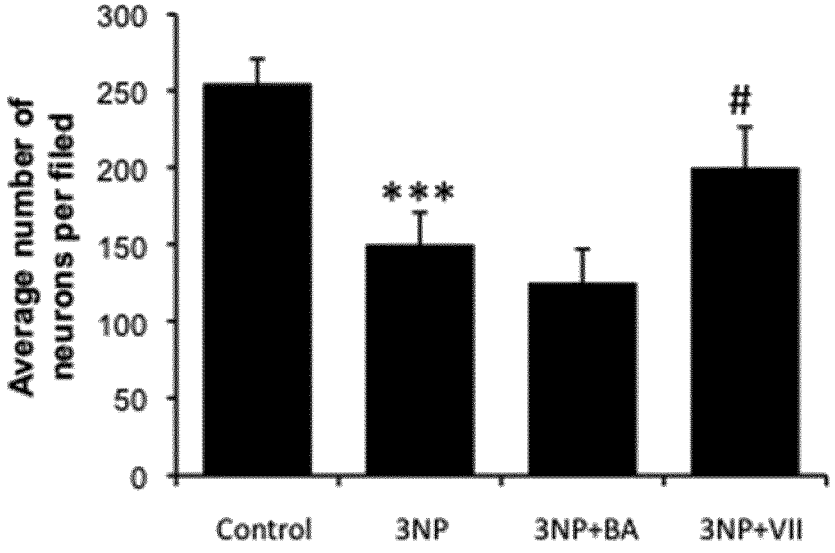

Average number of neurons per filed
300
250
200
150
100
50
0
***
#
Control
3NP
3NP+BA
3NP+VII

FIGURE 11

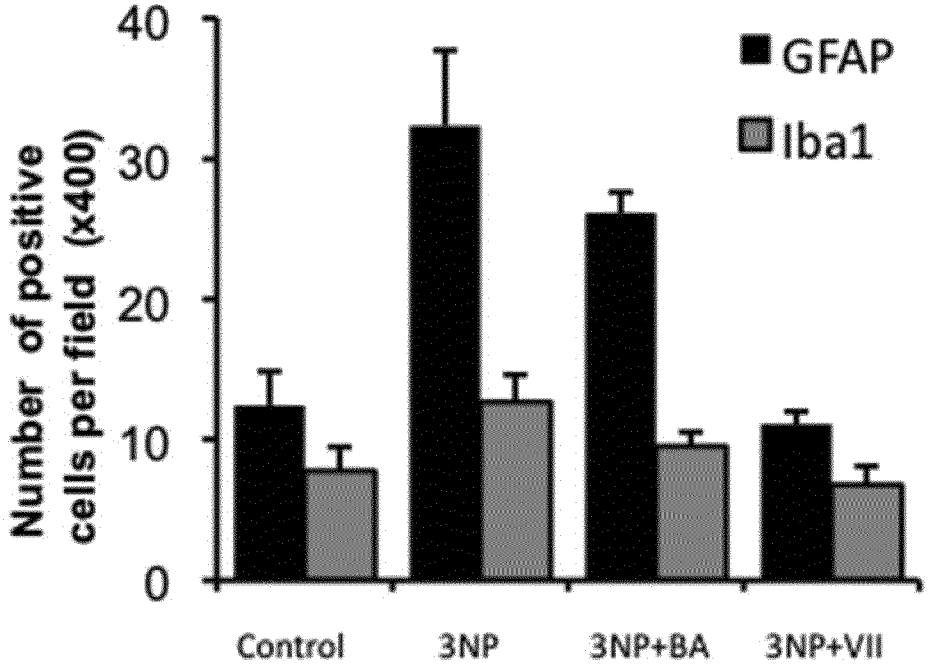
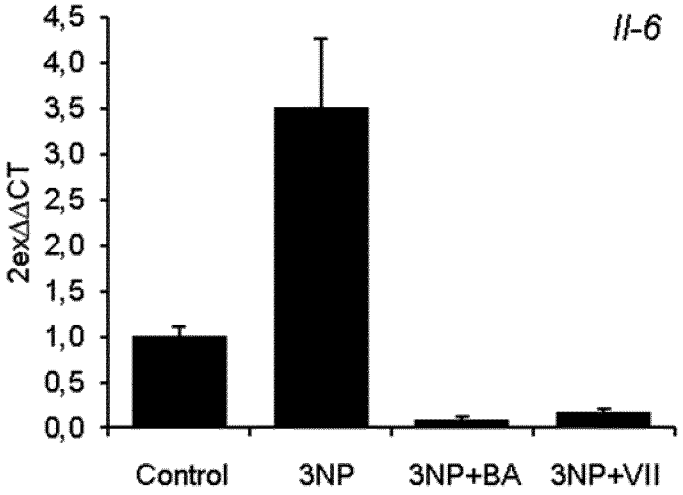

Number of positive cells per field (x400)
40
30
20
10
0
GFAP
Iba1
Control
3NP
3NP+BA
3NP+VII

FIGURE 12

12A



12B

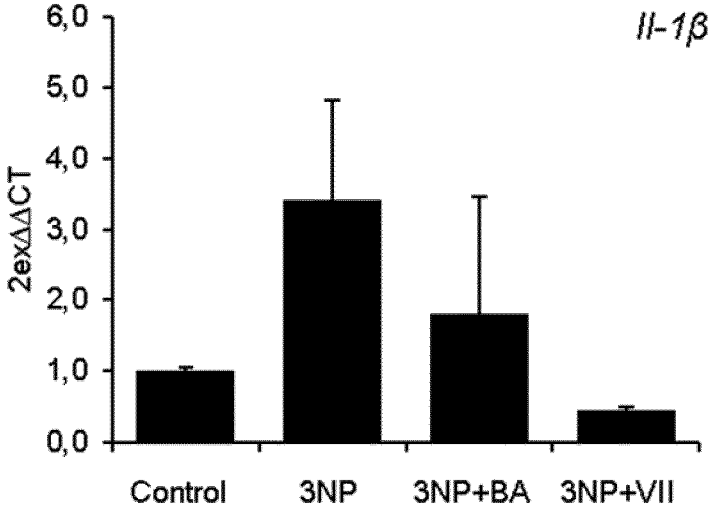
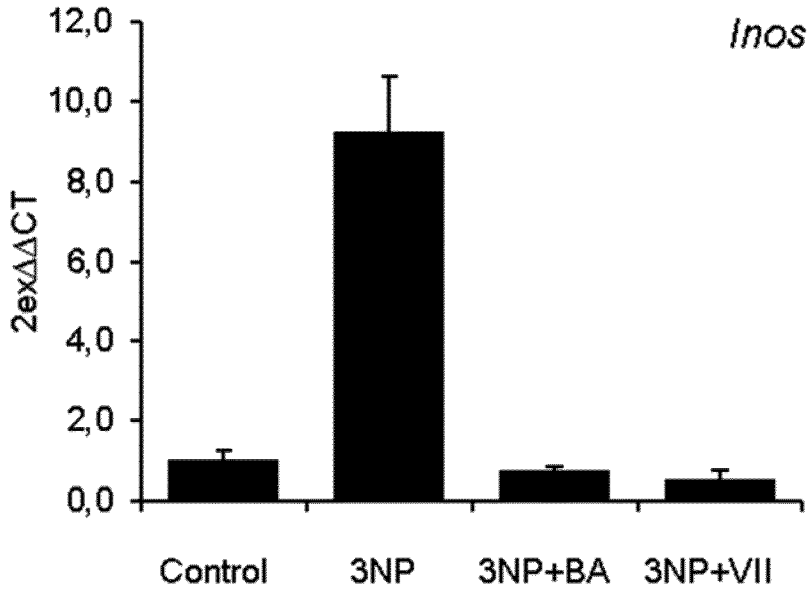


FIGURE 12 (cont.)

12C



12D

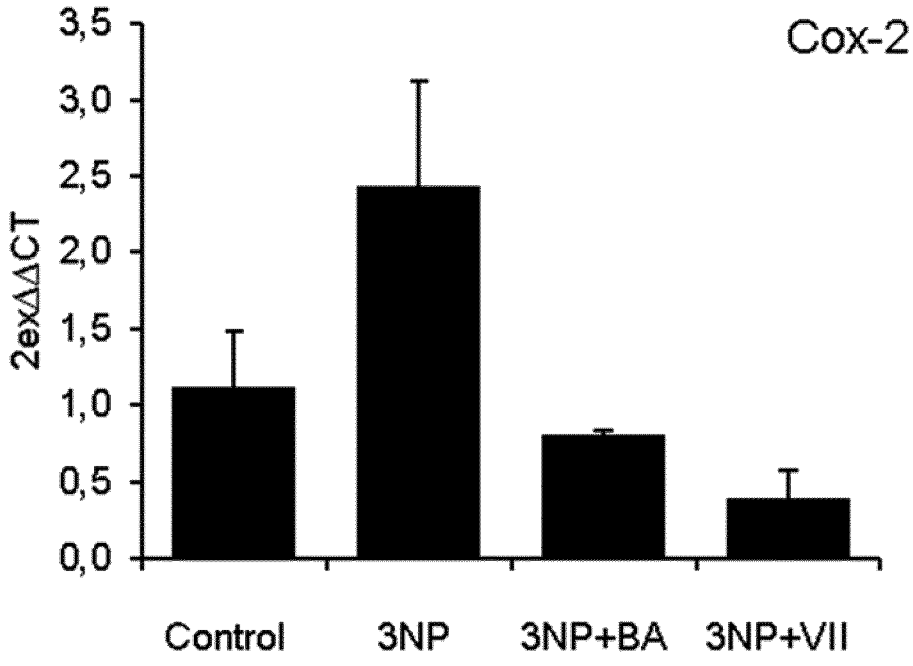
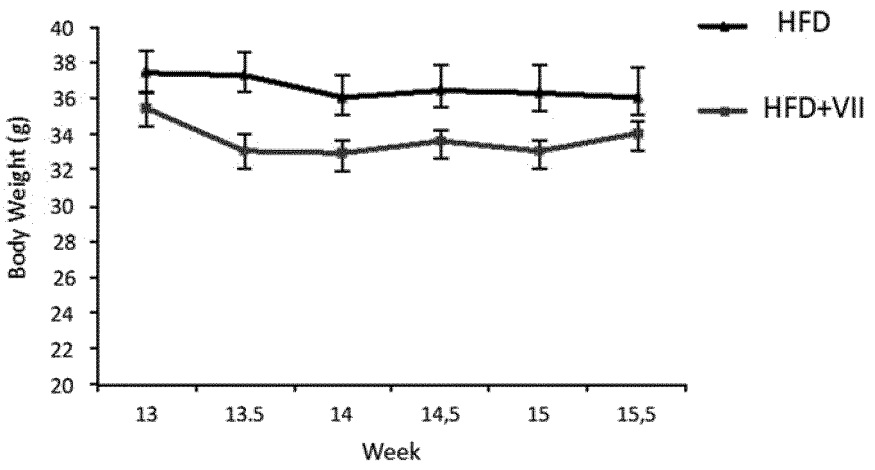
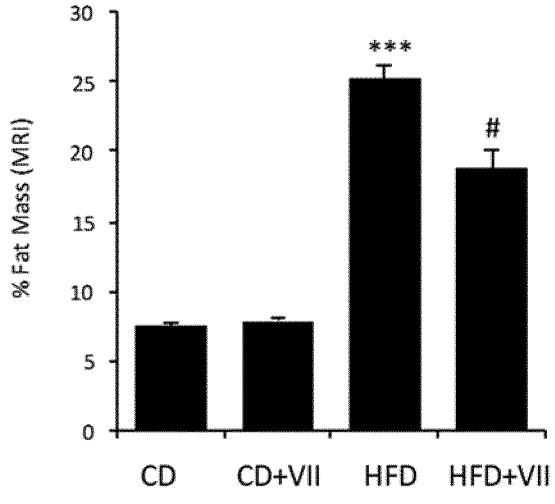


FIGURE 13

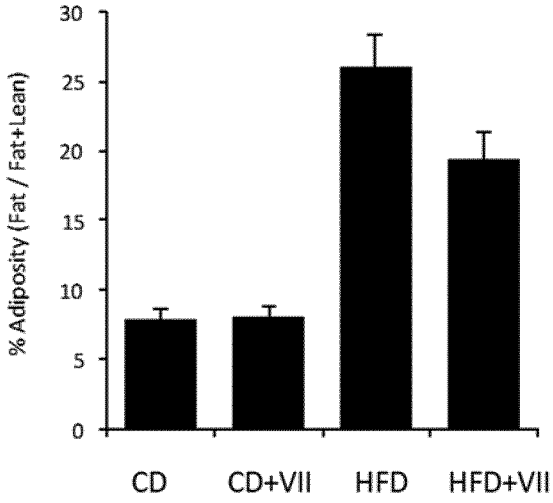


13A



13B

FIGURE 13 (cont.)



13C

FIGURE 14

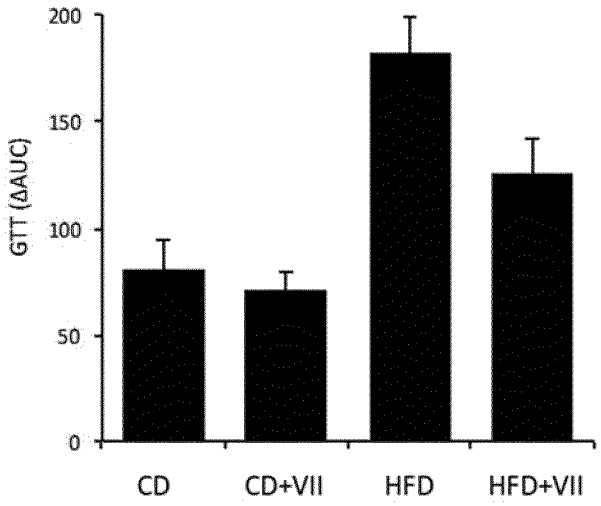
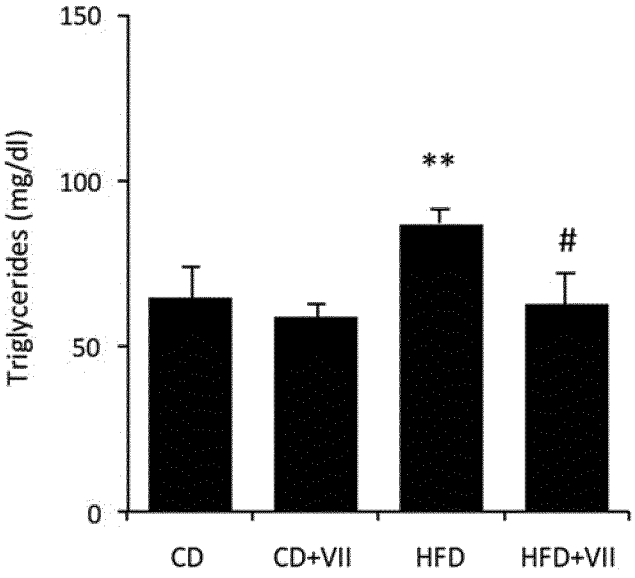


FIGURE 15



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/075042

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07J63/00 A61K31/575 A61P25/28 A61P9/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07J A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 8 324 264 B1 (ELDRIDGE GARY R [US] ET AL) 4 December 2012 (2012-12-04) column 19, lines 65-68 abstract	1-25
A	WIEMANN JANA ET AL: "Targeting cancer cells with oleanolic and ursolic acid derived hydroxamates", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 26, no. 3, 19 December 2015 (2015-12-19), pages 907-909, XP029391918, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2015.12.064 cited in the application page 908; compounds 2, 3, 15, 19 page 909; table 1	1-25

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 2018	30/01/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Watchorn, Peter

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JANA WIEMANN ET AL: "Betulinic acid derived hydroxamates and betulin derived carbamates are interesting scaffolds for the synthesis of novel cytotoxic compounds", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY., vol. 106, 1 December 2015 (2015-12-01), pages 194-210, XP055346496, FR ISSN: 0223-5234, DOI: 10.1016/j.ejmech.2015.10.043 cited in the application page 195; compounds 5, 6 page 197; table 1	1-25

A	EWA KARNA ET AL: "Betulinic acid inhibits the expression of hypoxia-inducible factor 1Î+- and vascular endothelial growth factor in human endometrial adenocarcinoma cells", MOLECULAR AND CELLULAR BIOCHEMISTRY, KLUWER ACADEMIC PUBLISHERS, BO, vol. 340, no. 1-2, 21 February 2010 (2010-02-21), pages 15-20, XP019826060, ISSN: 1573-4919 abstract	1-25

A	RONGXIN QI ET AL: "Oleanolic acid enhances the radiosensitivity of tumor cells under mimetic hypoxia through the reduction in intracellular GSH content and HIF-1[alpha] expression", ONCOLOGY REPORTS, vol. 31, no. 5, 6 March 2014 (2014-03-06), pages 2399-2406, XP055346676, ISSN: 1021-335X, DOI: 10.3892/or.2014.3064 abstract	1-25

A	TE-CHUN HSIA ET AL: "Maslinic Acid Induces Mitochondrial Apoptosis and Suppresses HIF-1[alpha] Expression in A549 Lung Cancer Cells under Normoxic and Hypoxic Conditions", MOLECULES, vol. 19, no. 12, 28 November 2014 (2014-11-28), pages 19892-19906, XP055346663, DOI: 10.3390/molecules191219892 abstract	1-25

	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SO YOUNG PARK ET AL: "Maslinic acid inhibits the metastatic capacity of DU145 human prostate cancer cells: possible mediation via hypoxia-inducible factor-1[alpha] signalling", BRITISH JOURNAL OF NUTRITION, vol. 109, no. 02, 13 April 2012 (2012-04-13), pages 210-222, XP055346679, UK ISSN: 0007-1145, DOI: 10.1017/S0007114512000967 abstract -----	1-25
A	SHAN JIAN-ZHEN ET AL: "Ursolic acid sensitized colon cancer cells to chemotherapy under hypoxia by inhibiting MDR1 through HIF-1[alpha]", ZHEJIANG UNIVERSITY. JOURNAL. SCIENCE B: INTERNATIONAL BIOMEDICINE & BIOTECHNOLOGY JOURNAL, ZHEIJIANG UNIVERSITY PRESS, CN, vol. 17, no. 9, 9 September 2016 (2016-09-09), pages 672-682, XP036049318, ISSN: 1673-1581, DOI: 10.1631/JZUS.B1600266 [retrieved on 2016-09-09] abstract -----	1-25
A	WO 2009/146216 A2 (REATA PHARMACEUTICALS INC [US]; JIANG XIN [US]; GREINER JACK; MARAVETZ) 3 December 2009 (2009-12-03) abstract page 84; compounds 402-9 pages 37-38; table 1 page 13, paragraph 3 page 14, last paragraph - page 15, paragraph 1 page 16, paragraph 2 page 17, paragraph 3 page 19, paragraph 1 page 42, paragraph 1 page 66, paragraph 1 -----	1-25
A	F. JUNG ET AL: "Hypoxic Regulation of Inducible Nitric Oxide Synthase via Hypoxia Inducible Factor-1 in Cardiac Myocytes", CIRCULATION RESEARCH., vol. 86, no. 3, 18 February 2000 (2000-02-18), pages 319-325, XP55440308, US ISSN: 0009-7330, DOI: 10.1161/01.RES.86.3.319 abstract -----	1-25

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/075042

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 8324264 B1	04-12-2012	US 8324264 B1	04-12-2012
		US 2013274256 A1	17-10-2013
WO 2009146216 A2	03-12-2009	TW 201004627 A	01-02-2010
		US 2010048911 A1	25-02-2010
		US 2012238767 A1	20-09-2012
		WO 2009146216 A2	03-12-2009

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2018.08.03

(21)申请号 201780004882.0

(22)申请日 2017.10.03

(30)优先权数据
16193684.4 2016.10.13 EP

(85)PCT国际申请进入国家阶段日
2018.06.19

(86)PCT国际申请的申请数据
PCT/EP2017/075042 2017.10.03

(87)PCT国际申请的公布数据
WO2018/069086 EN 2018.04.19

(71)申请人 维瓦赛尔生物技术西班牙有限公司
地址 西班牙科尔多瓦

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乔瓦尼·阿彭迪诺

(74)专利代理机构 中科专利商标代理有限责任公司 11021
代理人 吴小明

(51)Int.Cl.
C07J 63/00(2006.01)
A61K 31/575(2006.01)
A61P 25/28(2006.01)
A61P 9/10(2006.01)

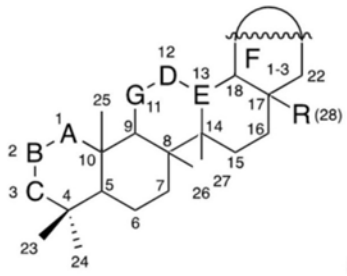
权利要求书11页 说明书47页
序列表3页 附图13页

(54)发明名称

氧肟酸三萜系化合物衍生物

(57)摘要

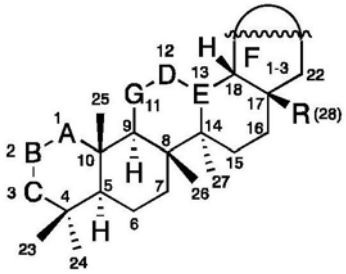
本发明记载了三萜系化合物和包含式(I)的三萜系化合物衍生物的组合物，其中R＝-C(O)NHOH。所述三萜系化合物和组合物表现出结合PHD2、稳定HIF-1α和HIF-2α蛋白、激活不同细胞类型中的HIF途径、诱导人内皮血管细胞中的血管发生、在体外和体内表现出神经保护活性、抗糖尿病活性和在体内降低脂质水平以及在体内提高红细胞生成素血浆水平的能力。所述三萜系化合物衍生物还以选择性方式作用并且不诱导Nrf2激活、NF-κB抑制、STAT3抑制和TGR5激活，这些是天然三萜系化合物前体的已知活性。所述三萜系化合物衍生物可用于治疗响应于HIF激活的病症和疾病，诸如卒中、脑瘫、创伤性损伤和神经变性疾病；以及IBD，心肌缺血-再灌损伤，急性肺损伤，糖尿病性和慢性伤口，器官移植，急性肾损伤或动脉病。



(I)

CN 108368150 A

1.式(Ia)的三萜衍生物或其立体异构体、药用盐或药用溶剂化物，



式(Ia)

其中独立地，

-A-B选自碳-碳单键或碳-碳双键；

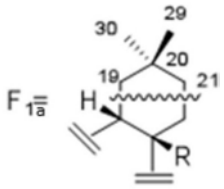
-B选自亚甲基(-CH_2-)，烯次甲基(=CH-)，羟基次甲基[-CH(OH)-]，或羟基化烯碳[-C(OH)=]；

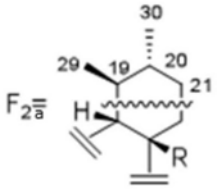
-B-C选自碳-碳单键或碳-碳双键；或是包含一个或多个杂原子的杂环的一部分，其中至少一个所述杂原子是氮；并且其中所述杂环是包含一个氮和一个氧的五元环；

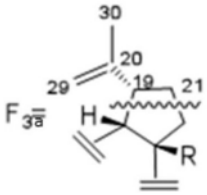
-C选自羟基次甲基[-CH(OH)-]，酰氧基次甲基[-CH(OCOR')-]，烯次甲基(=CH-)，羰基[-C(=O)-]，肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-]，其中R'是甲基；

-D-E是碳-碳单键或双键；

-F选自F_{1a}，F_{2a}或F_{3a}；







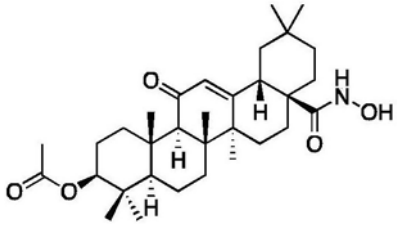
-G选自亚甲基(-CH_2-)或羰基[-C(=O)-]；并且

-R是氧肟酸基团(-CONHOH)；

并且其中，

-当C为酰氧基次甲基[-CH(OCOR')-]时，式(Ia)的三萜衍生物为

(V)



(3β)3-乙酰氧基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺;

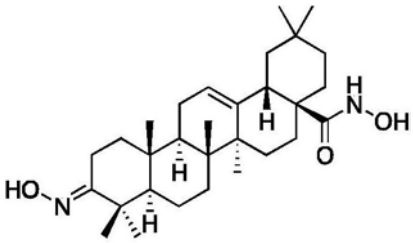
-当B为亚甲基(-CH_2-)时,C是羟基次甲基[-CH(OH)-],D-E是碳-碳双键,G是亚甲基(-CH_2-),并且R是氧肟酸基(-CONHOH),F是F_{3a};

-当B是亚甲基(-CH_2-)时,C是羟基次甲基[-CH(OH)-],D-E是碳-碳单键,G是亚甲基(-CH_2-),并且R是氧肟酸基(-CONHOH),F选自F_{1a}或F_{2a};

-当B是亚甲基(-CH_2-)时,C是肟[-C(=N-OH)-],D-E是碳-碳双键,G是亚甲基(-CH_2-),并且R是氧肟酸基(-CONHOH),F选自F_{1a}或F_{3a}。

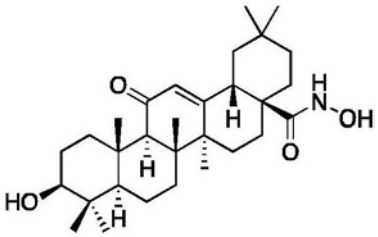
2.根据权利要求1所述的三萜衍生物,其中所述三萜衍生物选自:

(III)



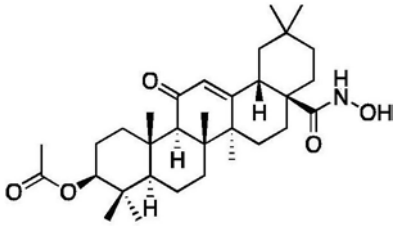
3-羟基亚胺基-N-羟基-齐墩果-12-烯-28-酰胺

(IV)

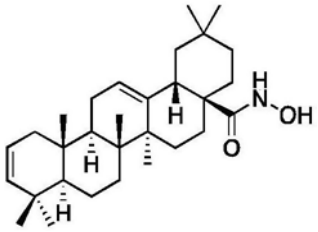


(3β)3-羟基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺

(V)

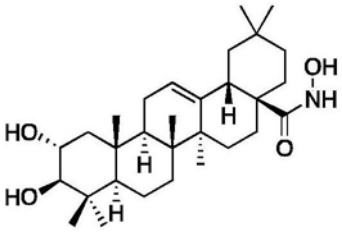


(3β)3-乙酰氧基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺(VI)



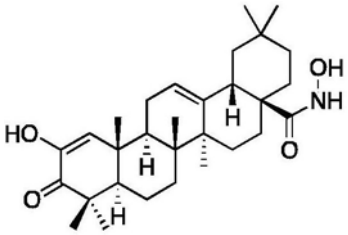
齐墩果-2,12-二烯-N-羟基-28-酰胺

(XIV)



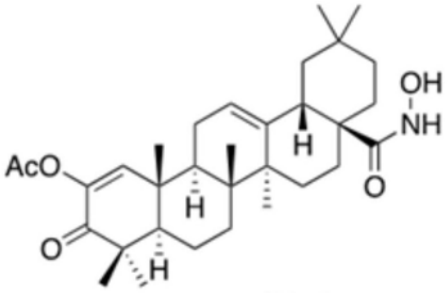
(2α,3β)2,3-二羟基-N-羟基-齐墩果-12-烯-28-酰胺

(XV)

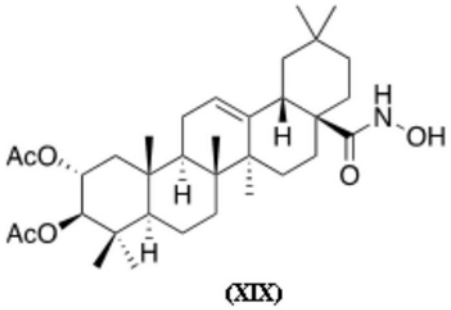


2-羟基-3-氧代-齐墩果-1,12-二烯-N-羟基-28-酰胺。

3.在合成权利要求1或2任一项的化合物中的中间体化合物,其选自XVIII或XIX:



(XVIII)



(XIX)

。

4.根据权利要求1或2任一项所述的式(Ia)的三萜衍生物,其用作药物。

5.根据权利要求1或2任一项所述的式(Ia)的三萜衍生物,其用于治疗响应于HIF途径激活的病症或疾病的应用。

6.根据权利要求5所述的用于应用的式(Ia)的三萜衍生物,其中所述响应于HIF途径激活的病症或疾病选自卒中、脑瘫、创伤性损伤和神经变性病。

7.根据权利要求5所述的用于应用的式(Ia)的三萜衍生物,其中所述响应于HIF途径激活的病症或疾病选自IBD、心肌缺血-再灌损伤、急性肺损伤、糖尿病性和慢性伤口、器官移植、急性肾损伤或动脉病。

8.一种药物组合物,其包含至少一种权利要求1-2中任一项所述的式(Ia)的三萜衍生物作为第一活性成分和至少一种赋形剂或载体,以及任选地还包含至少一种第二活性成分。

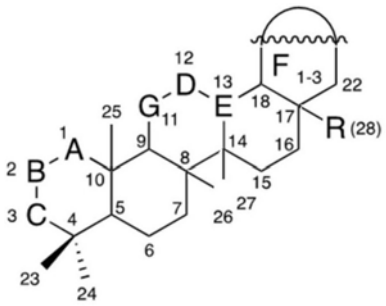
9.根据权利要求8所述的药物组合物,其用作药物。

10.根据权利要求8所述的药物组合物,其用于治疗响应于HIF途径激活的病症或疾病的应用。

11.根据权利要求10所述的用于应用的药物组合物,其中所述响应于HIF途径的激活的病症或疾病选自卒中、脑瘫、创伤性损伤和神经变性病。

12.根据权利要求10所述的用于应用的药物组合物,其中所述响应于HIF途径激活的病症或疾病选自IBD、心肌缺血-再灌损伤、急性肺损伤、糖尿病性和慢性伤口、器官移植、急性肾损伤或动脉病。

13.式(I)的三萜衍生物或其立体异构体、药用盐或药用溶剂化物,



式(I)

其中独立地,

-A-B选自碳-碳单键或碳-碳双键;

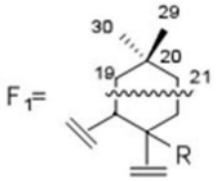
-B选自亚甲基(-CH_2-),烯次甲基(=CH-),羟基次甲基[-CH(OH)-],或羟基化烯碳[-C(OH)=];

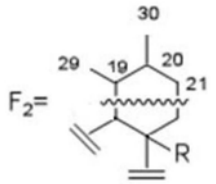
-B-C选自碳-碳单键或碳-碳双键;或是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮;并且其中所述杂环是包含一个氮和一个氧的五元环;

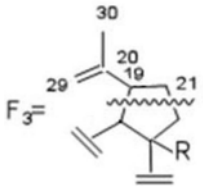
-C选自羟基次甲基[-CH(OH)-],酰氧基次甲基[-CH(OCOR')-],烯次甲基(=CH-),羰基[-C(=O)-],肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-],其中R'是甲基;

-D-E是碳-碳单键或双键;

-F选自F_1,F_2或F_3;







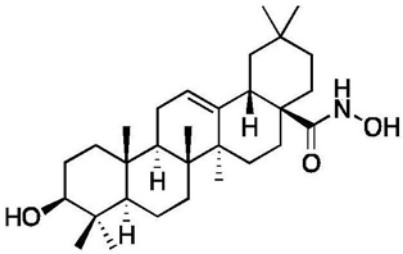
-G选自亚甲基(-CH_2-)或羰基[-C(=O)-];并且

-R是氧肟酸基团(-CONHOH);

其用于治疗响应于HIF途径激活的病症或疾病的应用。

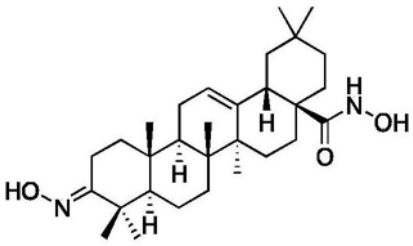
14.根据权利要求13所述的用于应用的三萜衍生物,其中所述三萜衍生物选自(II),(III),(IV),(V),(VI),(VII),(VIII),(X),(XI),(XII),(XIII),(XIV)或(XV):

(II)



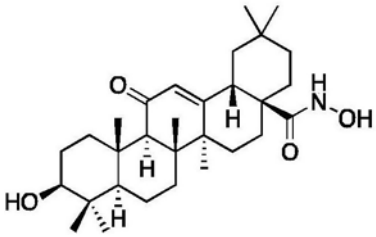
(3β)3-羟基-N-羟基-齐墩果-12-烯-28-酰胺

(III)



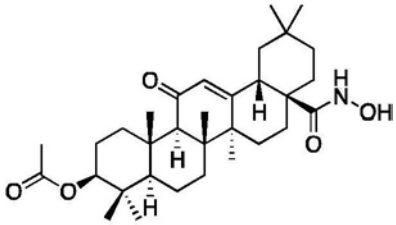
3-羟基亚胺基-N-羟基-齐墩果-12-烯-28-酰胺

(IV)



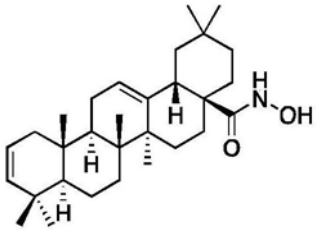
(3β)3-羟基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺

(V)



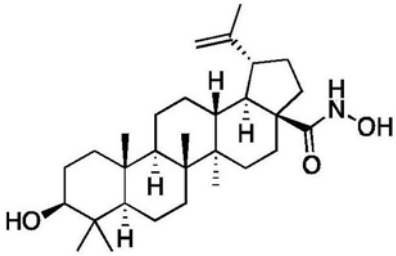
(3β)3-乙酰氧基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺

(VI)



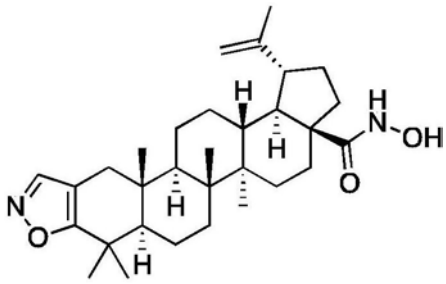
齐墩果-2,12-二烯-N-羟基-28-酰胺

(VII)



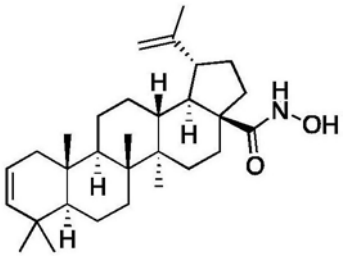
(3β)3-羟基-N-羟基-羽扇-20(29)-烯-28-酰胺

(VIII)



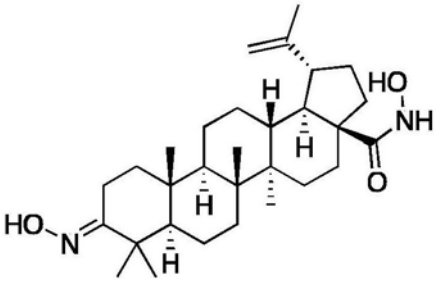
羽扇-2-烯并[2,3-d]异噁唑-N-羟基-28-酰胺

(X)



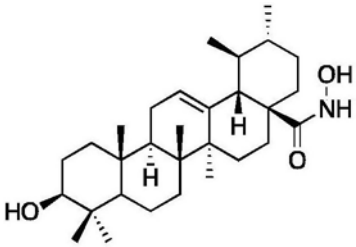
羽扇-2,20(29)-二烯-N-羟基-28-酰胺

(XI)



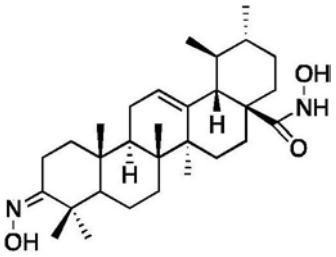
3-羟基亚胺基-N-羟基-羽扇-20(29)-烯-28-酰胺

(XII)



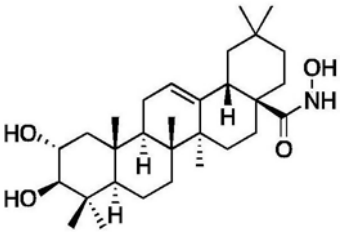
(3β)3-羟基-N-羟基-乌苏-12-烯-28-酰胺

(XIII)



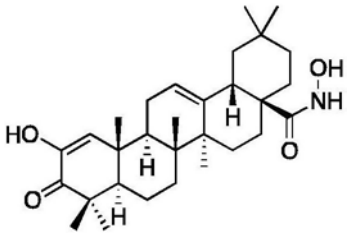
3羟基亚胺基-N-羟基-乌苏-12-烯-28-酰胺

(XIV)



(2α,3β)2,3-二羟基-N-羟基-齐墩果-12-烯-28-酰胺。

(XV)



2-羟基-3-氧代-齐墩果-1,12-二烯-N-羟基-28-酰胺。

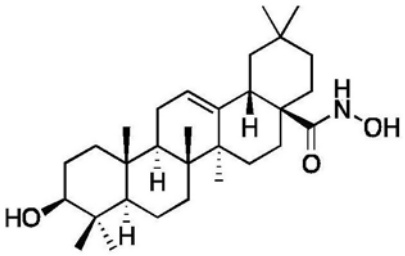
15.根据权利要求13或14所述的用于应用的三萜衍生物,其中所述响应于HIF途径激活的病症或疾病选自卒中、脑瘫、创伤性损伤和神经变性病。

16.根据权利要求13或14所述的用于应用的三萜衍生物,其中所述响应于HIF途径激活的病症或疾病选自IBD、心肌缺血-再灌损伤、急性肺损伤、糖尿病性和慢性伤口、器官移植、急性肾损伤或动脉病。

17.一种药物组合物,其包含至少一种权利要求13所述的式(I)的三萜衍生物或其立体异构体、药用盐或药用溶剂化物;和至少一种赋形剂或载体;所述药物组合物用于治疗响应于HIF途径激活的病症或疾病的应用。

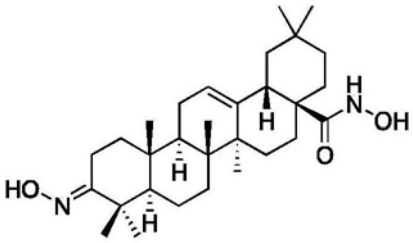
18.根据权利要求17所述的用于应用的药物组合物,其中所述至少一种三萜衍生物选自:

(II)



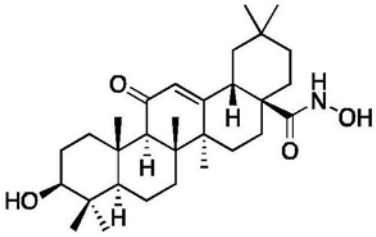
(3β)3-羟基-N-羟基-齐墩果-12-烯-28-酰胺

(III)



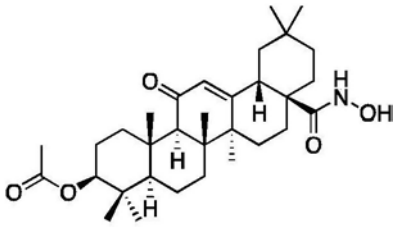
3-羟基亚胺基-N-羟基-齐墩果-12-烯-28-酰胺

(IV)



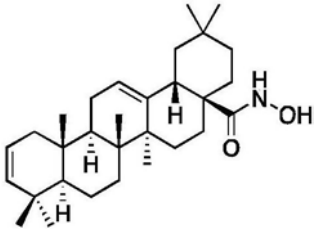
(3β)3-羟基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺

(V)



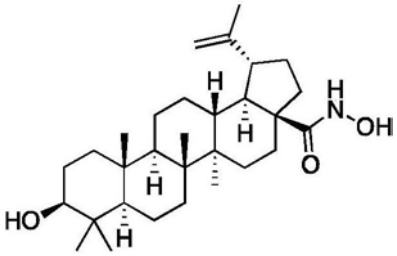
(3β)3-乙酰氧基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺

(VI)



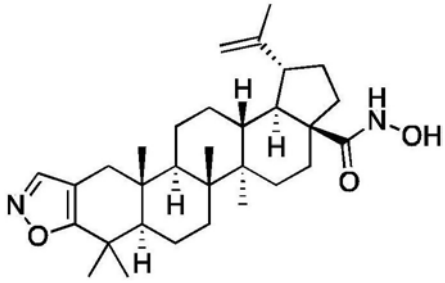
齐墩果-2,12-二烯-N-羟基-28-酰胺

(VII)



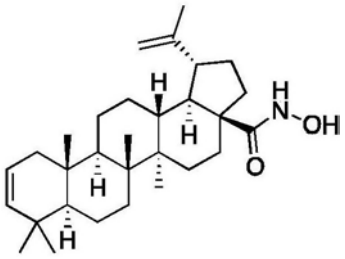
(3β)3-羟基-N-羟基-羽扇-20(29)-烯-28-酰胺

(VIII)



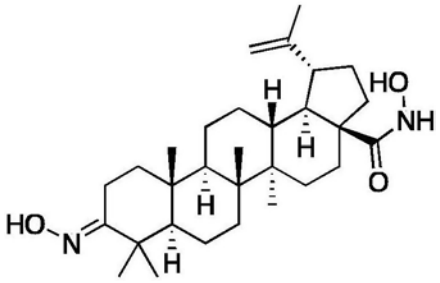
羽扇-2-烯并[2,3-d]异**噁**唑-N-羟基-28-酰胺

(X)



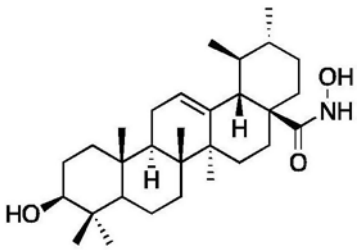
羽扇-2,20(29)-二烯-N-羟基-28-酰胺

(XI)



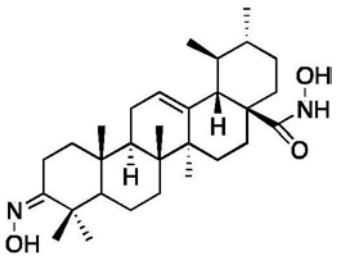
3-羟基亚胺基-N-羟基-羽扇-20(29)-烯-28-酰胺

(XII)



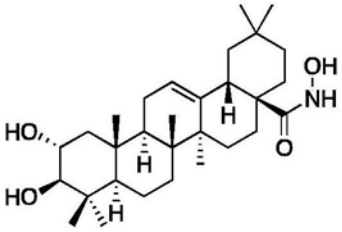
(3β)3-羟基-N-羟基-乌苏-12-烯-28-酰胺

(XIII)



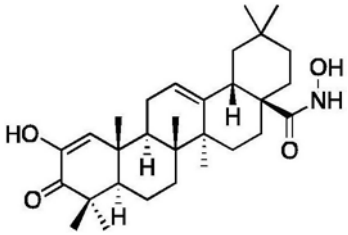
3羟基亚胺基-N-羟基-乌苏-12-烯-28-酰胺

(XIV)



(2α,3β)2,3-二羟基-N-羟基-齐墩果-12-烯-28-酰胺

(XV)



2-羟基-3-氧代-齐墩果-1,12-二烯-N-羟基-28-酰胺。

19.根据权利要求17或18中任一项所述的用于应用的药物组合物,其中所述响应于HIF途径激活的病症或疾病选自卒中、脑瘫、创伤性损伤和神经变性病。

20.根据权利要求17或18中任一项所述的用于应用的药物组合物，其中所述响应于HIF途径激活的病症或疾病选自IBD、心肌缺血-再灌损伤、急性肺损伤、糖尿病性和慢性伤口、器官移植、急性肾损伤或动脉病。

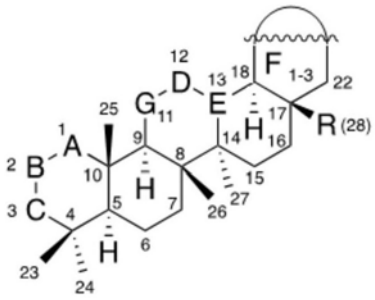
21.根据权利要求5所述的用于应用的式(Ia)的三萜衍生物，其中所述响应于HIF途径的病症或疾病独立地选自糖尿病、高脂血症或高甘油三酯血症。

22.根据权利要求10所述的用于应用的药物组合物，其中所述响应于HIF途径激活的病症或疾病独立地选自糖尿病、高脂血症或高甘油三酯血症。

23.根据权利要求13或14任一项所述的用于应用的式(I)的三萜衍生物，其中所述响应于HIF途径激活的病症或疾病独立地选自糖尿病、高脂血症或高甘油三酯血症。

24.根据权利要求17或18任一项所述的用于应用的药物组合物，其中所述响应于HIF途径激活的病症或疾病独立地选自糖尿病、高脂血症或高甘油三酯血症。

25.根据权利要求13所述的用于应用的式I的三萜衍生物，其中所述三萜衍生物是式(Ib)的三萜衍生物：



式(Ib)。

氧肟酸三萜系化合物衍生物

发明领域

[0001] 本发明涉及新型的三萜系化合物衍生物，以及所述化合物的合成。此外，本发明涉及其作为药物和在治疗中的用途，特别是作为HIF-1α和HIF-2α蛋白的稳定剂，HIF-1途径的激活剂，以及在治疗响应于HIF-1α和HIF-2稳定化的疾病和病症中的用途。本发明还提供包含所述化合物的药物组合物和使用所述化合物治疗疾病的方法。

[0002] 发明背景

[0003] 哺乳动物细胞需要维持恰当的氧稳态才能实现其需氧代谢和能量产生。自从发现了低氧诱导性因子(HIF)-1，便已经在生物学情形中广泛研究了作为由HIF转录因子引起的氧感知的基础的信号传导机制(Wang等人，Redox Rep.1996Apr.;2(2):89-96)。HIF由氧不稳定性α和组成型表达的β亚基组成，其驱动多种参与多样性细胞过程的基因的转录，所述多样性细胞过程包括红细胞生成、血管生成、能量代谢、缺血和炎症(Semenza等人，J.Biol.Chem.1994Sept 23;269(38):237357-63和Eltzschig等人，Nat.Rev.Drug Discov.2014Nov;13(11):852-69)。HIF几乎专门以两种形式存在于细胞中：HIF-1和HIF-2。它们是异型二聚体转录因子，在HIF-1和HIF-2的情形中，分别由组成型产生的高丰度HIF-β亚基和HIF-1α或HIF-2α配偶体组成，具有48％的序列同源性(Rabinowitz MH，J.Med.Chem.2013Dec 12;56(23):9369-4025)。HIF-1通常与针对低氧的代谢和血管响应相关，而HIF-2与血管系统相关，但也稍微更与红细胞生成相关(Ratcliffe PJ，J.Clin.Invest.2007Apr;117(4):862-5)。

[0004] 氧控制HIF-1α的机制已经通过鉴定HIF脯氨酰羟化酶(PHD)得到揭示(Bruick和McKnight，Science 2001Nov 9;294(5545):1337-40)。在含氧量正常(normoxia)时，PHD使HIF-1α氧依赖性降解(ODD)结构域中的脯氨酸残基羟基化，由此允许与VHL蛋白(Hippel Lindau protein，pVHL)-延伸蛋白B-延伸蛋白C结合，导致活性遍在蛋白化和半衰期约为5分钟的降解。另一方面，低氧下氧缺乏消弱PHD对HIF-1α的羟基化，产生还原的HIF-1α、周转(turnover)和随后诱导靶基因转录(Rabinowitz MH，J.Med.Chem.2013Dec 12;56(23):9369-4025)。PHD属于双加氧酶家族，它们的催化活性需要氧、铁和2-氧戊二酸(2-OG)。它们针对氧的低亲和力是生理氧浓度的约2至10倍高，这允许该酶作用为氧传感物。已经鉴定了三种PHD同种型(PHD1，PHD2和PHD3)，并且已知它们的底物是相当多样性的和同种型-特异性的(Rabinowitz MH，J.Med.Chem.2013Dec 12;56(23):9369-4025；和Eltzschig等人，Nat.Rev.Drug Discov.2014Nov;13(11):852-69)。

[0005] 认为PHD2在调节HIF途径中是关键的。具体而言，在条件性敲除PHD2时观察到增强的血管生成和提高的血管内皮生长因子(VEGF)-A和红细胞生成素(EPO)水平(Takeda等人，Circulation 2007Aug.14;116(7):774-81)。这些观察以及之前的HIF增加EPO释放和同时增加红细胞生成的报道暗示：通过调节PHD激活HIF可能对患有贫血和缺血相关疾病的患者有益。因此，已经寻求通过抑制PHD活性激活HIF途径的药理学方式来治疗系统和局部疾病，所述疾病的治疗可以得益于HIF激活(Rabinowitz MH，J.Med.Chem.2013Dec 12;56(23):9369-4025；和Eltzschig等人，Nat.Rev.Drug Discov.2014Nov;13(11):852-69)。

[0006] 低氧和炎症在多个水平上密切相关，并且在多种人类疾病中具有功能作用。事实上，宽泛的临床病症特征在于低氧-或缺血-驱动的炎症或炎症相关的低氧。日益增多的证据表明，炎症损伤特征在于发生组织低氧，其可能是增加的代谢和减少的氧供应的结果。例如，这是在患有炎性肠病(inflammatory bowel disease，IBD)的患者中观察到的肠道炎症过程中的情形，所述炎性肠病包括溃疡性结肠炎(ulcerative colitis)和局限性肠炎(Crohn's disease)(Karhausen等人，J.Clin Invest.2004Oct;114(8):1098-106)。此类炎性低氧由代谢供应和需求率的明显变化引起。新出现的证据表明，在组织低氧过程中同时抑制PHD和随后稳定HIF能够起作用作为内源性适应反应，以抵消低氧-驱动的炎症和恢复正常的细胞功能(Eltzschig等人，Nat.Rev.Drug Discov.2014Nov;13(11):852-69)。

[0007] 续发于诸如大脑缺氧的脑损伤和神经变性过程的神经损害是涉及炎性变化的复杂过程。在这些病症中已经观察到由HIF-1α的稳定和反式激活介导的与存活或细胞死亡相关的普通机制的激活。PHD是HIF-1α的氧依赖性降解的看门人，并且还起作用作为细胞代谢的集成传感物(Aragonés等人，Cell Metab.2009Jan 7;9(1):11-22)。大约二十年前发现了低氧预处理(hypoxic preconditioning，HP)保护免于后续更严重的缺氧(anoxia)的现象。之后，已经在体外和体内急性低氧模型中透彻研究了HP的作用。尽管还没有完全公开准确的机制，但是已经推断了潜在的分子机制。例如，HP激活很多种内源性保护介质(包括HIF-1α和HIF-2α)稳定化增加了在严重的氧缺乏下细胞存活的能力(Wu等人，2012，Theprotective role of hypoxic preconditioning in CNS(CNS中低氧预处理的保护作用)，Anoxia，Dr.Pamela Padilla(Ed.)，InTech，DOI:10.5772/27621)。

[0008] 存在低氧的脑病主要包括卒中(stroke)、脑瘫(cerebral palsy)、创伤性损伤等。直到现在，尚没有任何有效的药物保护脑免受这些疾病。HP机制的公开将有助于预防所述疾病的药物发现。多种针对低氧的细胞适应性反应通过HIF-1α介导，并且HP对该因子的激活增强耐受严重缺氧或缺血的能力。另一方面，HIF-1的靶基因参与能量稳态，诸如调节红细胞生成中的EPO，血管生成中的血管内皮生长因子(VEGF)，葡萄糖摄入中的葡萄糖传递物(GLUT)和无氧酵解的酵解酶(Speer等人，Free Radio.Biol.Med.2013Sep;62:23-36)。并且，已经报道，在少突胶质细胞中激活HIF-1α诱导EPO，其在少突胶质细胞中赋予针对兴奋性中毒(excitotoxicity)的保护作用(Sun等人，J.Neurosci.2010Jul 14;30(28):9621-30)。在这一意义上，也已经证明了EPO在诸如多发性硬化(Multiple Sclerosis)、卒中和创伤性脑损伤的一些疾病中的益处(Peng等人，J.Neurosurg.2014Sep;121(3):653-64;Ehrenreich等人，Stroke 2009Dec;40(12):e647-56;Li等人，Ann.Neurol.2004Dec;56(6):767-77)。另外，低氧模拟剂(即，产生的生物反应与发生低氧时产生的生物反应相同的药剂)，诸如去铁胺(desferrioxamine，DFX)防护由3-硝基丙酸(3-nitropoionic acid)诱导的神经元损伤(Yang等人，J.Neurochem.2005May;96(3):513-25)。因此，低氧模拟性小分子对PHD的抑制作用代表一种令人感兴趣的策略或用于存在低氧的病症的临床管理的神经保护性疗法的开发，所述存在低氧的病症诸如卒中，脑瘫，创伤性损伤和神经变性疾病，诸如多发性硬化、亨廷顿病(Huntington disease)、阿尔茨海默病(Alzheimer disease)和帕金森病(Parkinson disease)。

[0009] 已经在动物模型中进行了大量的药理学研究(通常使用非特异性PDH2抑制剂)，并且已经进行了一些临床研究。事实上，一些公司正参与用于贫血和其他适应证的PHD抑制剂

的发现和研发,其他适应证如IBD,心肌缺血-再灌损伤,急性肺损伤,器官移植,急性肾损伤和动脉疾病,它们是许多研究者积极追求PHD抑制剂作为新型治疗方法的领域(Rabinowitz MH,J.Med.Chem.2013Dec 12;56(23):9369-4025;和Eltzschig等人,Nat.Rev.Drug Discov.2014Nov;13(11):852-69)。

[0010] HIF-选择性PHD作为HIF表达的调节剂的初始记载已经为开发基于PHD的分子工具和疗法提供了模板。通过2-OG类似物使PHD药理学失活足以稳定HIF-1α,但是这一作用就个体PHD同种型来说是非特异性的,并且体外研究表明HIF1α的ODD序列被PHD2最有效地羟基化(Rabinowitz MH,J.Med.Chem.2013Dec 12;56(23):9369-4025)。这些观察已经对鉴定修饰酶的小分子抑制剂产生了相当大的兴趣。事实上,已经记载了一些PHD抑制剂种类,包括铁螯合剂,如DFX,肼苯哒嗪(hydralazine),AKB?4924,FG?2229,TM?6008和l?mimosine;CUL2deneddylators,如MLN4924;2-OG模拟物,如ximethyloxalylglycine和N-草酰-d-苯丙氨酸;PHD活性位点阻断剂,如吡唑并吡啶(pyrazolopyridines),8?羟基喹啉,化合物A,FG?4497和TM?6089;和Fe^{2+}取代基,如Co^{2+},Ni^{2+}和Cu^{2+}。这些化合物的作用机制是基于这样的观察:共底物2-OG与携带必需的Fe^{2+}离子的催化结构域的结合对于酶PHD2活性是极其重要的。因此,在结构上模拟2-OG的化合物,如N-草酰甘氨酸或其前体DMOG,通过阻断共底物的进入而抑制PHD2(Rabinowitz MH,J.Med.Chem.2013Dec 12;56(23):9369-4025;和Eltzschig等人,Nat.Rev.Drug Discov.2014Nov;13(11):852-69)。

[0011] DFX是氧肟酸(hydroxamic acid)衍生物,其作用为金属螯合剂,在临床应用上用于治疗急性铁中毒和由于输血依赖性贫血(transfusion-dependent anemias)导致的慢性铁超负荷。然而,没有标明DFX用于治疗慢性疾病,并且,主要由于肾脏排泄所述药物和铁螯合物,其在严重的肾病中是禁忌使用的。并且,在细胞中,DFX仅在相对高的浓度诱导HIF-1α稳定和激活。

[0012] 三萜系化合物广泛地分布在可食用的和药用的植物中,并且是人饮食的固有部分。其中,五环三萜系化合物由直链烃角鲨烯衍生,并且它们是高度多功能的,并且由此在农业、食品、化妆品和药物领域内具有宽泛的商业应用,作为杀虫剂、药物、辅剂、抗微生物剂、抗癌剂、表面活性剂、防腐剂等(Sheng等人,Nat.Prod.Rep.2011Mar;28(3):543-93;Yadav等人,Toxins(Basel)20100ct;2(10):2428-66)。三萜系化合物的酸性官能团和羟基(-OH)基团不能以固定相相互作用,原因在于这两个基团位于该化合物的相对侧。存在三个主要的三萜家族:齐墩果烷(oleane)、乌苏烷和羽扇烷三萜。在齐墩果烷家族中存在的主要三萜系化合物是齐墩果酸(oleanolic acid)、山楂酸、高根二醇和β-香树素;在乌苏烷家族中存在的是熊果酸和熊果醇;并且在羽扇烷家族中存在的是羽扇醇、桦木醇和桦木酸(Yadav等人,Toxins(Basel)20100ct;2(10):2428-66)。

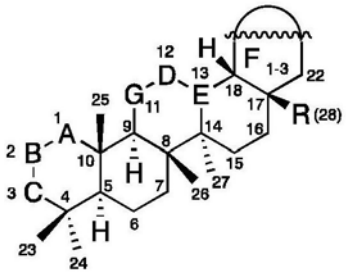
[0013] 已经广泛研究了饮食三萜系化合物,如齐墩果酸、熊果酸、桦木酸和山楂酸的生物活性和药物应用,并且它们也被广泛用作模板来制备具有提高的生物活性的新型衍生物。已表明三萜系化合物抑制转录因子NF-κB(细胞核因子-κB)和STAT3(信号转导及转录激活蛋白3)的激活,调节Nrf2(NFE2L2或细胞核因子(红细胞(erythroid)-来源的2)-样2)途径,并且结合和激活胆汁受体TGR5(Sato等人,Biochem.Biophys.Res.Comm.2007Nov 3;362(4):793-8)。然而,还没有记载三萜系化合物对PDH2抑制和HIF-1α稳定化的潜在活性。令人

惊讶地，熊果酸、桦木酸和齐墩果酸(它们是本文公开的化合物II至XIV的前体)结合PHD2，但是不激活HIF-1α途径。这一发现导致产生氧肟酸(hydroxamate)衍生物，其能够结合PHD2，螯合在其活性口袋中的Fe^{2+}，并且因此这些化合物激活HIF途径并稳定HIF-1α与HIF-2α蛋白。化合物III，IV，V，VI，VIII，IX，X，XI，和XIV以及XV是新型的化学实体，而已报道化合物II，VII，XII和XIII在体外具有抗癌活性，其不与HIF-1α激活相关(Wiemann等人，Eur.J.Med.Chem.2015Dec 1;106:194-210;Wiemann等人，Bioorg.Med.Chem.Lett.2016Feb 1;26(3):907-9;CN102180939B)。事实上，已经提示一些三萜系化合物的HIF-1α抑制作用(与HIF-1α激活相反)作为抗癌疗法的潜在策略(Dasgupta等人，Angiogenesis.2015Jul;18(3):283-99;Jin等人，Arch Pharm Res.2007Apr;30(4):412-8;Dai等人，J Nat Prod.2006Dec;69(12):1715-20)。

[0014] 简述

[0015] 不同于现有技术，本发明的问题是提供诱导HIF-1α和HIF-2α稳定化并激活HIF途径的新型五环三萜系化合物衍生物(三萜系化合物衍生物)。

[0016] 本发明涉及式(Ia)的三萜衍生物或立体异构体、药用盐或药用溶剂化物，

[0017] 

式(Ia)

[0018] 其中独立地，

[0019] -A-B选自碳-碳单键或碳-碳双键；

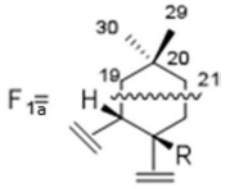
[0020] -B选自亚甲基(-CH_2-)，烯次甲基(=CH-)，羟基次甲基[-CH(OH)-]，或羟基化烯碳[-C(OH)=]；

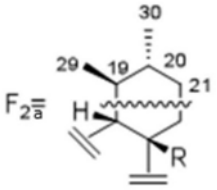
[0021] -B-C选自碳-碳单键或碳-碳双键；或是包含一个或多个杂原子的杂环的一部分，其中至少一个所述杂原子是氮；并且其中所述杂环是包含一个氮和一个氧的五元环；

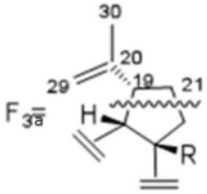
[0022] -C选自羟基次甲基[-CH(OH)-]，酰氧基次甲基[-CH(OCOR')-]，烯次甲基(=CH-)，羰基[-C(=O)-]，肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-]，其中R'是甲基；

[0023] -D-E是碳-碳单键或双键；

[0024] -F选自F_{1a}，F_{2a}或F_{3a}；



[0025] 

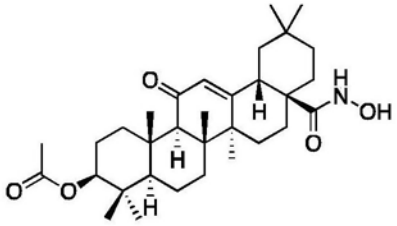


[0026] -G选自亚甲基(-CH_2-)或羰基[-C(=O)-];和

[0027] -R是氧肟酸基团(-CONHOH);

[0028] 并且其中

[0029] -当C为酰氧基次甲基[-CH(OCOR')-]时,式(Ia)的三萜衍生物为(V)

[0030] 

(3β) 3-乙酰氧基-11-氧代-齐墩果-12-烯-*N*-羟基-28-酰胺;

[0031] -当B为亚甲基(-CH_2-)时,C是羟基次甲基[-CH(OH)-],D-E是碳-碳双键,G是亚甲基(-CH_2-),并且R是氧肟酸基(-CONHOH),F是F_{3a};

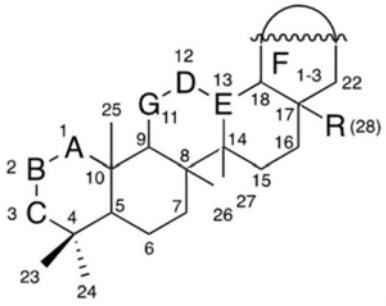
[0032] -当B是亚甲基(-CH_2-)时,C是羟基次甲基[-CH(OH)-],D-E是碳-碳单键,G是亚甲基(-CH_2-),并且R是氧肟酸基(-CONHOH),F选自F_{1a}或F_{2a};

[0033] -当B是亚甲基(-CH_2-)时,C是肟[-C(=N-OH)-],D-E是碳-碳双键,G是亚甲基(-CH_2-),并且R是氧肟酸基(-CONHOH),F选自F_{1a}或F_{3a}。

[0034] 另外,本发明涉及包含至少一种作为第一活性成分的如本文上文所述的式(Ia)的三萜衍生物和至少一种赋形剂或载体的药物组合物,并且任选地还包含至少一种第二活性成分。

[0035] 本发明的另一方面涉及如本文上文所述的式(Ia)的三萜衍生物及其药物组合物,其用作药物。

[0036] 本发明还涉及式(I)的三萜衍生物或其立体异构体、药用盐或药用溶剂化物,

[0037] 

式(I)

[0038] 其中独立的，

[0039] -A-B选自碳-碳单键或碳-碳双键；

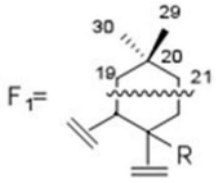
[0040] -B选自亚甲基(-CH_2-)，烯次甲基(=CH-)，羟基次甲基[-CH(OH)-]，或羟基化烯碳[-C(OH)=]；

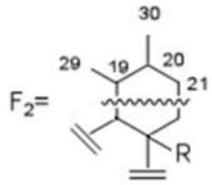
[0041] -B-C选自碳-碳单键或碳-碳双键；或是包含一个或多个杂原子的杂环的一部分，其中至少一个所述杂原子是氮；并且其中所述杂环是包含一个氮和一个氧的五元环；

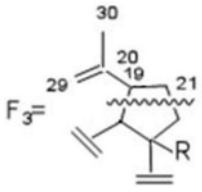
[0042] -C选自羟基次甲基[-CH(OH)-]，酰氧基次甲基[-CH(OCOR')-]，烯次甲基(=CH-)，羰基[-C(=O)-]，肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-]，其中R'是甲基；

[0043] -D-E是碳-碳单键或双键；

[0044] -F选自F_1，F_2或F_3；



[0045] 



[0046] -G选自亚甲基(-CH_2-)或羰基[-C(=O)-]；并且

[0047] -R是氧肟酸基(-CONHOH)；

[0048] 其用于治疗响应于HIF途径的激活的病症或疾病的应用。

[0049] 本发明还涉及包含本文上文所述的三萜衍生物的药物组合物，其用于治疗响应于HIF途径的激活的病症或疾病。

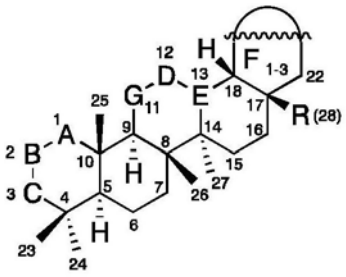
[0050] 发明详述

[0051] 本发明涉及在位置28包含氧肟酸官能团的三萜系化合物衍生物和包含所述三萜

系化合物衍生物的组合物，其表现出结合PHD2、稳定HIF-1α和HIF-2α蛋白、激活不同细胞类型中的HIF途径、诱导人内皮血管细胞中的血管发生、在体外和体内表现出神经保护活性、体内抗糖尿病活性和在体内提高红细胞生成素血浆水平的能力。

[0052] 所述三萜系化合物衍生物还以选择性方式作用并且不诱导Nrf2激活、NF-κB抑制、STAT3抑制和TGR5激活，这些是它们天然的三萜系化合物前体的已知活性。所述三萜系化合物衍生物可用于治疗响应于HIF途径激活的病症和或疾病。因此，本发明涉及其治疗受益于或响应于HIF-1α和HIF-2α激活的疾病，并且因此，本发明不涉及其治疗受益于或响应于HIF-1α抑制的疾病或病症。

[0053] 更具体地，本发明涉及式(Ia)的三萜衍生物：

[0054] 

式(Ia)

[0055] 其中独立地，

[0056] -A-B选自碳-碳单键或碳-碳双键；

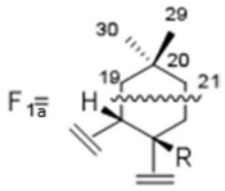
[0057] -B选自亚甲基(-CH_2-)，烯次甲基(=CH-)，羟基次甲基[-CH(OH)-]，或羟基化烯碳[-C(OH)=]；

[0058] -B-C选自碳-碳单键或碳-碳双键；或是包含一个或多个杂原子的杂环的一部分，其中至少一个所述杂原子是氮；

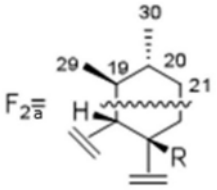
[0059] -C选自羟基次甲基[-CH(OH)-]，酰氧基次甲基[-CH(OCOR')-]，烯次甲基(=CH-)，羰基[-C(=O)-]，肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-]，其中R'是甲基；

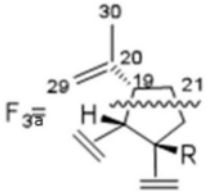
[0060] -D-E是碳-碳单键或双键；

[0061] -F选自F_{1a}，F_{2a}和F_{3a}；



[0062]





[0063] -G选自亚甲基(-CH_2-)或羰基[-C(=O)-];并且

[0064] -R是氧肟酸基(-CONHOH);

[0065] 并且其中

[0066] -当B是亚甲基(-CH_2-)时,C是羟基次甲基[-CH(OH)-],D-E是碳-碳双键,并且R是氧肟酸基(-CONHOH),F是F_{3a};

[0067] -当B是亚甲基(-CH_2-)时,C是羟基次甲基[-CH(OH)-],D-E是碳-碳单键,并且R是氧肟酸基(-CONHOH),F选自F_{1a}或F_{2a};

[0068] -当B是亚甲基(-CH_2-)时,C是肟[-C(=N-OH)-],D-E是碳-碳双键,并且R是氧肟酸基(-CONHOH),F选自F_{2a}或F3a。

[0069] 如本文上文公开的,在式(Ia)的三萜衍生物的一个实施方案中,B-C可以是包含一个或多个杂原子、其中至少一个所述杂原子是氮的杂环的一部分,并且其中所述杂环是五元环。

[0070] 如本文上文公开的,在式(Ia)的三萜衍生物的一个实施方案中,B-C可以是包含一个或多个杂原子、其中至少一个所述杂原子是氮的杂环的一部分,并且其中所述杂环是包含两个氮原子的五元环。

[0071] 如本文上文公开的,在式(Ia)的三萜衍生物的一个实施方案中,B-C可以是包含一个或多个杂原子、其中至少一个所述杂原子是氮的杂环的一部分,并且其中所述杂环是包含一个氮和一个氧的五元环。

[0072] 如本文上文公开的式(Ia)的三萜衍生物还包括其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物。

[0073] 就本说明书的目的而言,术语“一种/多种类似物”是指与本发明公开的化合物结构上衍生的或同源的任意实体。

[0074] 在本发明的情况下,术语本文公开的化合物的“一种/多种衍生物”应该解释为按

照本文上文R的定义总是在位置28取代的任意三萜系化合物类似物，其为所述三萜系化合物化合物提供本文所述的药理学特性，但是特征也在于在三萜系化合物分子其他位置的其他不同于本文所述的取代的取代。

[0075] 术语“互变异构体”是通过化学过程(互变异构)容易互变的有机化合物的结构异构体。

[0076] 术语″异构体″由IUPAC(国际纯粹和应用化学协会(International Union of Pure and Applied Chemistry))正式定义为数个种类(或分子实体)中的一个，所述种类(或分子实体)具有相同的原子组成(分子式)但是具有不同的分子式或不同的立体化学式，而″立体异构体″由IUPAC正式定义为这样的化合物，所述化合物具有相同的化学组成，但是关于原子或基团的空间排列不同。

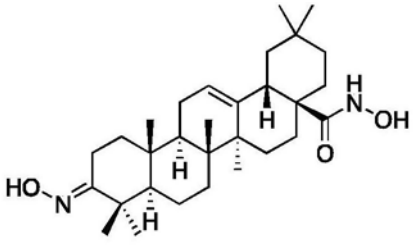
[0077] 用于本文时，“多晶型物”是指具有相同化学组成，但形成晶体的分子、原子和/或离子的空间排列不同的晶体。

[0078] 术语″药用盐″是指任意这样的药用盐，其在施用给患者后能够(直接或间接地)提供如本文中所述的化合物。这样的盐优选为与生理学可接受的有机或无机酸的酸加成盐。酸加成盐的实例包括无机酸加成盐，如，例如，盐酸盐、氢溴酸盐、氢碘酸盐、硫酸盐、硝酸盐、磷酸盐，和有机酸加成盐，如，例如，乙酸盐、三氟乙酸盐、马来酸盐、富马酸盐、柠檬酸盐、草酸盐、琥珀酸盐、酒石酸盐、苹果酸盐、扁桃酸盐、甲磺酸盐和对甲苯磺酸盐。碱加成盐的实例包括无机盐，如，例如，钠盐、钾盐、钙盐和铵盐，和有机碱盐，如，例如，乙二胺盐、乙醇胺盐、N,N-二亚烷基乙醇胺盐、三乙醇胺盐和碱性氨基酸盐。然而，应该理解，非药用盐也落在本发明的范围内，因为那些可以在药用盐的制备中有用。盐形成的步骤在本领域中是常规的。

[0079] 根据本发明，术语″溶剂化物″应该理解为意为根据本发明的任何形式的活性化合物，其中所述化合物通过非共价键与另一分子(通常是极性溶剂)键合，其特别包括水合物和醇合物。

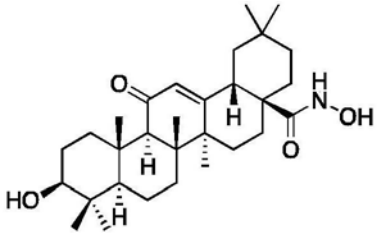
[0080] 在优选的实施方案中，所公开的三萜衍生物是指式(III)，(IV)，(V)，(VI)，(VIII)，(IX)，(X)，(XI)，(XIV)，(XV)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物。

[0081] (III)

[0082] 

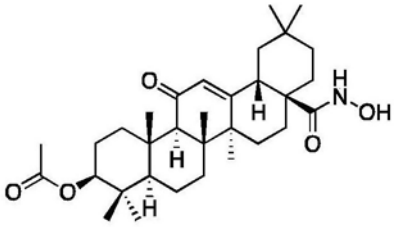
3-羟基亚胺基-*N*-羟基-齐墩果-12-烯-28-酰胺

[0083] (IV)

[0084] 

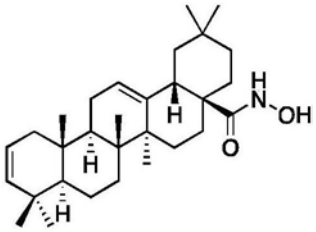
(3β) 3-羟基-11-氧代-齐墩果-12-烯-*N*-羟基-28-酰胺

[0085] (V)

[0086] 

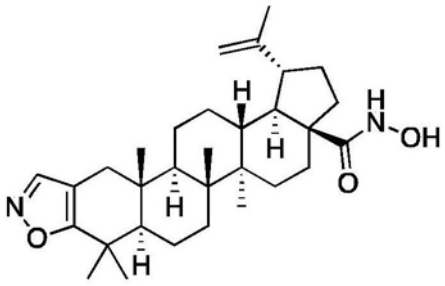
(3β) 3-乙酰氧基-11-氧代-齐墩果-12-烯-*N*-羟基-28-酰胺

[0087] (VI)

[0088] 

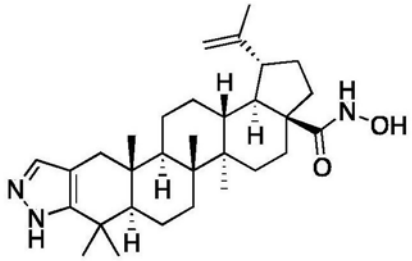
齐墩果-2,12-二烯-*N*-羟基-28-酰胺

[0089] (VIII)

[0090] 

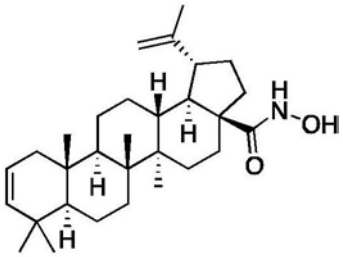
羽扇-2-烯并[2,3-d]异噁唑-*N*-羟基-28-酰胺

[0091] (IX)

[0092] 

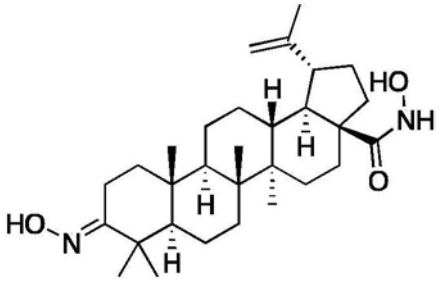
1'H-羽扇-20(29)-烯并[3,2-c]吡唑-*N*-羟基-28-酰胺

[0093] (X)

[0094] 

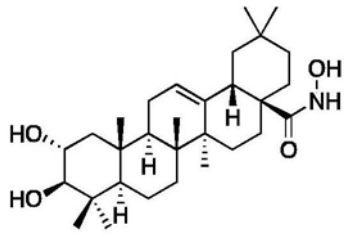
羽扇-2,20(29)-二烯-*N*-羟基-28-酰胺

[0095] (XI)

[0096] 

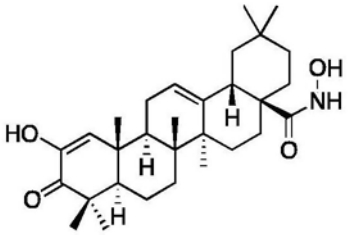
3-氧代-羽扇-20(29)-烯-28-酸

[0097] (XIV)

[0098] 

(2α,3β) 2,3-二羟基-*N*-羟基-齐墩果-12-烯-28-酰胺

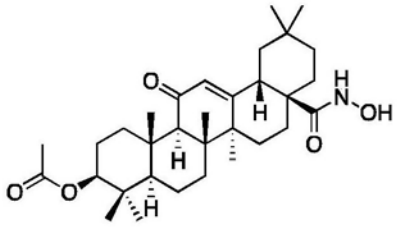
[0099] (XV)

[0100] 

2-羟基-3-氧代-齐墩果-1,12-二烯-*N*-羟基-28-酰胺

[0101] 在式(Ia)的三萜衍生物的优选的实施方案中，当C是酰氧基次甲基[-CH(OCOR')-]时，式(Ia)的三萜衍生物是

[0102] (V)

[0103] 

(3β) 3-乙酰氧基-11-氧代-齐墩果-12-烯-*N*-羟基-28-酰胺；

[0104] 如在本发明的实施例和附图中所示，通式(Ia)中包括的修饰赋予本文公开的化合物下述能力：结合PHD2、稳定HIF-1α和HIF-2α蛋白、激活不同细胞类型中的HIF途径、诱导人内皮血管细胞中的血管发生、在体外和体内表现出神经保护活性、体内抗糖尿病活性和在体内提高红细胞生成素血浆水平。所述的式(Ia)化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物以选择性方式作用，并且不诱导Nrf2激活、NF-κB抑制、STAT3抑制和TGR5激活，这些是它们天然的三萜系化合物前体的已知活性。

[0105] 本文公开的一个实施方案涉及组合物，特别是药物组合物，其至少包含如本文上文所述的式(Ia)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物，以及至少一种赋形剂或载体。

[0106] 就本发明的目的而言，所述赋形剂或载体是指惰性成分，诸如，但不限于，共溶剂，表面活性剂，油，保湿剂，润滑剂，防腐剂，稳定剂和抗氧化剂。可以使用任意的药理学可接受的缓冲液，诸如TRIS或任意磷酸盐缓冲液。

[0107] 本文公开的另一个实施方案涉及组合物，特别是药物组合物，其至少包含如本文上文所述的式(Ia)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物，以及至少一种具有加和或增效的生物学活性的第二活性化合物。

[0108] 就本发明的目的而言，术语“活性化合物或活性成分”应为被认为是同义的，并且意为当施用给人或动物时发挥疗效的化学实体。

[0109] 本文公开的另一个实施方案涉及本发明式(Ia)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物，其用作药物。

[0110] 本文公开的另一个实施方案涉及本文以上所述的包含本发明式(Ia)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物的药物组合物，其用作药物。

[0111] 所述式(Ia)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物表现出下述能力：结合PHD2，稳定HIF-1α和HIF-2α蛋白，激活不同细胞类型中的HIF途径，诱导人内皮血管细胞中的血管发生，在体外和体内表现出神经保护活性，体内抗糖尿病活性，并且在体内提高红细胞生成素血浆水平。所述式(Ia)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物以选择性方式作用并且不诱导Nrf2激活、NF-κB抑制、STAT3抑制和TGR5激活，这些是它们天然的三萜系化合物前体的已知活性。

[0112] 所述式Ia的三萜系化合物衍生物及其药物组合物可用于治疗响应于HIF途径的激活的病症和疾病。

[0113] 因此，式(Ia)的三萜衍生物可用于响应于HIF途径的激活的病症或疾病，其中由此，所述病症或疾病的治疗受益于或响应于HIF途径的激活。

[0114] 根据本发明，响应于HIF途径的激活的病症和疾病不包括实体瘤。情况是这样的，因为实体瘤发展新生血管生成，其促进转移扩散，因此研发HIF-1抑制剂作为抗癌药，从而阻断异常的血管生长和功能(Potente等人Cell.2011Sep 16;146(6):873-87)。

[0115] 具体地，一个实施方案公开本文上述的式(Ia)的三萜衍生物及其药物组合物，其用于治疗各种各样的响应于HIF途径的激活的病症或疾病，所述病症或疾病包括，但不限于，卒中，脑瘫，创伤性损伤和神经变性疾病。神经变性疾病的实例包括，但不限于，多发性硬化，亨廷顿病，阿尔茨海默病(Alzheimer disease)或帕金森病(Parkinson disease)。

[0116] 就本说明书的目的而言，卒中是指脑血管病因的神经缺陷。卒中可以归类为两大类：缺血和出血。缺血性卒中由对脑血液供应的中断引起，而出血性卒中由血管破裂或异常的血管结构导致。

[0117] 就本说明书的目的而言，脑瘫是指一组永久性的运动障碍，其在幼童时期出现并且由控制运动、平衡和姿势的脑部分的异常发育或损害引起。

[0118] 就本说明书的目的而言，创伤性损伤是指突然发作和重度的物理损伤，其需要立即的医学护理。创伤性损伤是广泛多样的钝伤(blunt)、穿透伤和烧伤机制的结果。所述创伤性损伤包括机动车辆碰撞、运动损伤、摔倒、自然灾害和多种其他的物理损伤。

[0119] 另一个实施方案公开了本文上文所述的式(Ia)的三萜衍生物及其药物组合物，其用于治疗各种各样的响应于HIF途径激活的病症或疾病，其中所述病症或疾病包括，但不限于，IBD，心肌缺血-再灌损伤，急性肺损伤，糖尿病性和慢性伤口，器官移植，急性肾损伤或动脉病。更优选地，所述病症或疾病选自IBD，心肌缺血-再灌损伤，急性肺损伤，器官移植，急性肾损伤或动脉病。

[0120] 就本说明书的目的而言，IBD是指一组结肠和小肠的炎性病症。局限性肠炎和溃疡性结肠炎特别是炎性肠病的主要类型。

[0121] 就本说明书的目的而言，心肌缺血-再灌损伤是指当在心肌缺血或缺氧(缺氧、低氧)一段时间后对组织恢复血液供应时引起的组织损伤。

[0122] 就本说明书的目的而言，急性肺损伤是指这样的病症，其以急性严重的低氧为特

征，并且其诊断是基于病危患者中存在非心源性肺水肿和呼吸衰竭。

[0123] 就本说明书的目的而言，传染病是指由病原性微生物如细菌、病毒、寄生虫或真菌引起的疾病，并且其中所述疾病可以直接或间接地从一个人扩散到另一个人。

[0124] 就本说明书的目的而言，糖尿病性和慢性伤口是指这样的伤口，其可能由糖尿病病症引起，其不以大部分伤口愈合的方式在有序的一组阶段和可预期量的时间内愈合。一般来说，通常认为在三个月内没有愈合的伤口是慢性的。

[0125] 就本说明书的目的而言，急性肾损伤是指在7日内发展的由多种因素引起的突然的肾功能丧失。所述突然的肾功能丧失引起对肾组织的损害并且其通常由减小的肾血流(肾缺血)引起。

[0126] 就本说明书的目的而言，动脉疾病包括一类涉及血管的疾病。

[0127] 具体而言，一个实施方案公开本文所述的式(Ia)的化合物及其药物组合物，其用于治疗响应于HIF途径激活的病症或疾病，所述病症或疾病选自IBD，心肌缺血-再灌损伤，急性肺损伤，糖尿病性和慢性伤口，器官移植，急性肾损伤或动脉病。在一个优选的实施方案中，所述响应于HIF途径激活的病症或疾病选自IBD，心肌缺血-再灌损伤，急性肺损伤，器官移植，急性肾损伤或动脉病。

[0128] 本发明的一个实施方案还公开了本文上文所述的式(Ia)的化合物及其药物组合物，其用于治疗响应于HIF途径激活的病症或疾病，其中所述响应于HIF途径激活的病症或疾病是代谢病理学。在一个优选的实施方案中，所述代谢病理学是糖尿病。在另一个优选的实施方案中，所述代谢病理学是脂质代谢病症。在另一个优选的实施方案中，所述代谢病理学是高脂血症(hyperlipidemia)。在另一个优选的实施方案中，所述代谢病理学是高甘油三酯血症(hypertriglyceridemia)。已经记载PHD2抑制剂改善葡萄糖和脂质代谢并且保护免于肥胖和代谢综合征(Rahtu-Korpela等人，Diabetes.2014Oct;63(10):3324-33)。

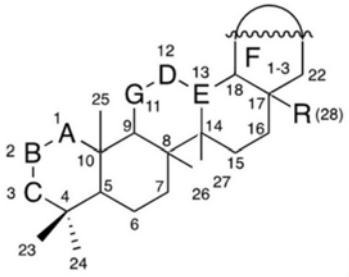
[0129] 术语“代谢病理学”是指其中正常的代谢过程已经改变的病理学。

[0130] 就本发明的目的而言，术语“糖尿病”是指一组在延长时间内存在高血糖水平的代谢病症。糖尿病是由于胰腺的低胰岛素产量或由于机体对所产生的胰岛素的不恰当的反应导致。

[0131] “高脂血症”是指这样的病症，其中受试者血液中存在异常升高水平的任意或全部的脂质或脂蛋白。

[0132] “高甘油三酯血症”是指这样的病症，其中受试者存在高血液水平的甘油三酯。升高水平的甘油三酯与动脉粥样硬化(atherosclerosis)相关，甚至在不存在高胆固醇血症(hypercholesterolemia)(高胆固醇水平)时也相关，并且倾向于引起心血管疾病。非常高的甘油三酯水平还增加急性胰腺炎的危险。

[0133] 本文公开的另一个实施方案是指式(I)的三萜衍生物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物，

[0134] 

式(I)

[0135] 其中独立地，

[0136] -A-B选自碳-碳单键或碳-碳双键；

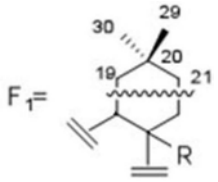
[0137] -B选自亚甲基(-CH_2-)，烯次甲基(=CH-)，羟基次甲基[-CH(OH)-]，或羟基化烯碳[-C(OH)=]；

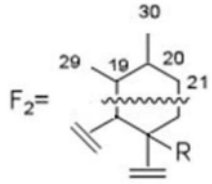
[0138] -B-C选自碳-碳单键或碳-碳双键；或是包含一个或多个杂原子的杂环的一部分，其中至少一个所述杂原子是氮；

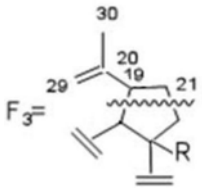
[0139] -C选自羟基次甲基[-CH(OH)-]，酰氧基次甲基[-CH(OCOR')-]，烯次甲基(=CH-)，羰基[-C(=O)-]，肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-]，其中R'是甲基；

[0140] -D-E是碳-碳单键或双键；

[0141] -F选自F_1，F_2或F_3；



[0142] 



[0143] -G选自亚甲基(-CH_2-)或羰基[-C(=O)-]；并且

[0144] -R是氧肟酸基(-CONHOH)；

[0145] 其用于治疗响应于HIF途径激活的病症或疾病。

[0146] 因此，式(I)的三萜衍生物可用于响应于HIF途径激活的病症或疾病，其中因此，所述病症或疾病的治疗受益于或响应于HIF途径的激活。

[0147] 如本文上文公开的式(I)的三萜衍生物还包含本文上文就本发明的目的而言定义的其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物。具体地，本文上文公开的本发明式(I)的三萜衍生物包含其所有的立体异构体和任意

的药用盐或药用溶剂化物。

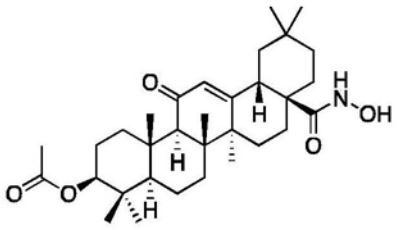
[0148] 在本文上文公开的式(I)的三萜衍生物的一个实施方案中,B-C可以是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮,并且其中所述杂环是五元环。

[0149] 在本文上文公开的式(I)的三萜衍生物的一个实施方案中,B-C可以是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮,并且其中所述杂环是包含两个氮原子的五元环。

[0150] 在本文上文公开的式(I)的三萜衍生物的一个实施方案中,B-C可以是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮,并且其中所述杂环是包含一个氮和一个氧的五元环。

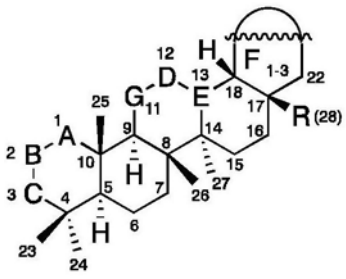
[0151] 在式(I)的三萜衍生物的另一个实施方案中,当C是酰氧基次甲基[-CH(OCOR')-]时,式(I)的三萜衍生物为

[0152] (V)

[0153] 

(3β) 3-乙酰氧基-11-氧代-齐墩果-12-烯-*N*-羟基-28-酰胺;

[0154] 式(I)的三萜衍生物的一个实施方案是指式(Ia)所示的其立体异构体:

[0155] 

式(Ia)

[0156] 其中独立地

[0157] -A-B选自碳-碳单键或碳-碳双键;

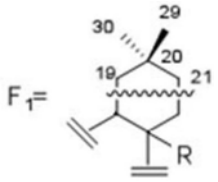
[0158] -B选自亚甲基(-CH_2-),烯次甲基(=CH-),羟基次甲基[-CH(OH)-],或羟基化烯碳[-C(OH)=];

[0159] -B-C选自碳-碳单键或碳-碳双键;或是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮;

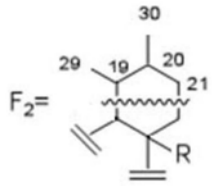
[0160] -C选自羟基次甲基[-CH(OH)-],酰氧基次甲基[-CH(OCOR')-],烯次甲基(=CH-),羰基[-C(=O)-],肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-],其中R'是甲基;

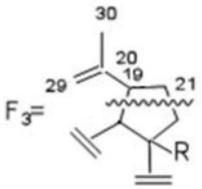
[0161] -D-E是碳-碳单键或双键;

[0162] -F选自

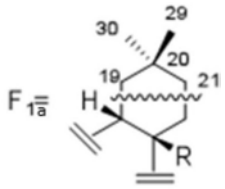


[0163]

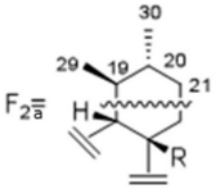


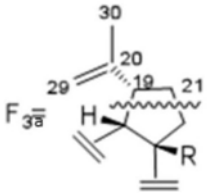


[0164] 并且其中F_1是F_{1a},F_2是F_{2a}并且F_3是F_{3a};



[0165]



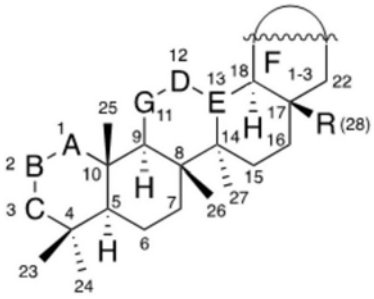


[0166] -G选自亚甲基(-CH_2-)或羰基[-C(=O)-];并且

[0167] -R是氧肟酸基(-CONHOH);

[0168] 其用于治疗响应于HIF途径激活的病症或疾病。

[0169] 式(I)的三萜衍生物的另一个实施方案是指式(Ib)所示的其立体异构体:

[0170] 

式(Ib)

[0171] 其中独立地，

[0172] -A-B选自碳-碳单键或碳-碳双键；

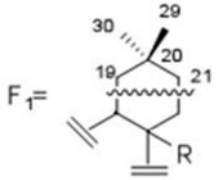
[0173] -B选自亚甲基(-CH_2-)，烯次甲基(=CH-)，羟基次甲基[-CH(OH)-]，或羟基化烯碳[-C(OH)=]；

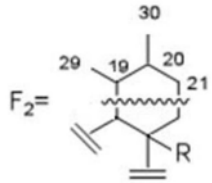
[0174] -B-C选自碳-碳单键或碳-碳双键；或是包含一个或多个杂原子的杂环的一部分，其中至少一个所述杂原子是氮；

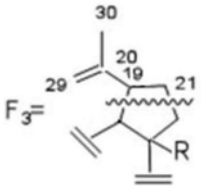
[0175] -C选自羟基次甲基[-CH(OH)-]，酰氧基次甲基[-CH(OCOR')-]，烯次甲基(=CH-)，羰基[-C(=O)-]，肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-]，其中R'是甲基；

[0176] -D-E是碳-碳单键或双键；

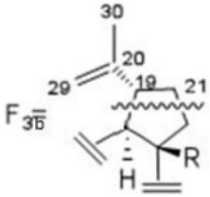
[0177] -F选自F_1，F_2或F_3；



[0178] 



[0179] 并且其中当F是F_3时，F_3是F_{3b}：

[0180] 

[0181] 其用于治疗响应于HIF途径激活的病症或疾病。

[0182] 本发明所述的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物表现出下述能力：

[0183] -结合PHD2，如下文公开的实施例2所示，

[0184] -稳定HIF-1α和HIF-2α蛋白，如下文公开的实施例4所示，

[0185] -激活不同细胞类型中的HIF途径，如下文公开的实施例3所示，

[0186] -诱导人内皮血管细胞中的血管发生，如下文公开的实施例5所示，

[0187] -在体外和体内表现出神经保护活性，如下文公开的实施例7所示，并且

[0188] -在体内提高红细胞生成素血浆水平，如下文公开的实施例6所示；

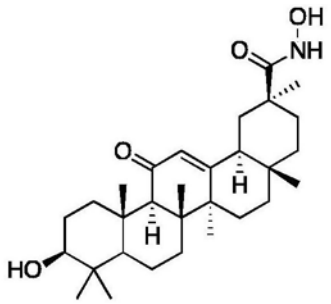
[0189] -表现出抗糖尿病活性和在体内降低脂质水平，如下文公开的实施例9所示。

[0190] 所述三萜系化合物衍生物还以选择性方式作用，并且不诱导Nrf2激活、NF-κB抑制、STAT3抑制和TGR5激活，这些是它们天然的三萜系化合物前体的已知活性(如在本发明的实施例3中所示)。

[0191] 如上文提及的，本发明的实施例3表明式(I)的化合物在不同细胞型中怎样激活HIF途径。实施例3还检测了两种比较化合物XX和CDDO-ME。

[0192] 比较化合物XX，衍生自甘草亭酸(glycyrrethinic acid)，其在位置20包含氧肟酸基，并且因此不像本发明的化合物那样在位置28包含氧肟酸基。

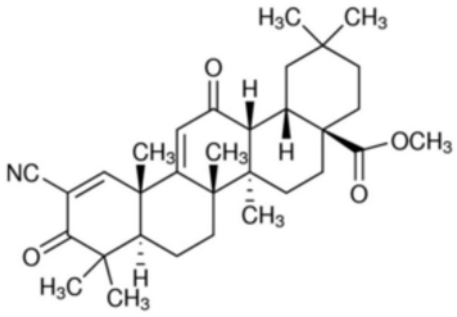
[0193] (XX)

[0194] 

(3β) 3-羟基-11-氧代-齐墩果-12-烯-*N*-羟基-29-酰胺

[0195] 实施例3的结果表明：氧肟酸基的位置从本发明化合物的特征位置28的变化导致不存在HIF途径的激活。

[0196] 另一方面，比较化合物CDDO-Me(2-氰基-3,12-二氧代-齐墩果-1,9(11)-二烯-28-酸甲酯，CAS编号218600-53-4)是齐墩果酸衍生物，但是其在位置2包含氰基(在式I的位置B的取代基)，该取代基是与在本发明公开的式I的化合物的所述位置特有的取代基基本上不同的取代基。

[0197] 

CDDO-Me

[0198] 实施例3的结果表明:CDDO-Me不提供HIF途径的激活,并且因此,不具有式I化合物的结构特征的比较化合物XX和CDDO-Me都不提供HIF途径的激活。

[0199] 因此,本发明公开的式I定义的三萜系化合物衍生物可用于治疗响应于HIF途径的激活的病症或疾病,即,其治疗受益于HIF-1α和HIF-2α激活的病症或疾病。

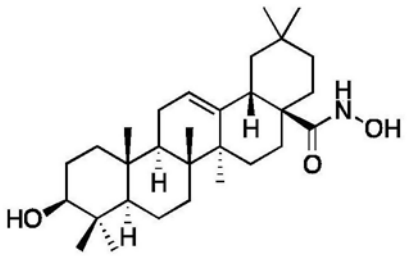
[0200] 具体而言,一个实施方案公开上文所述的式(I)的三萜衍生物,其用于治疗响应于HIF途径激活的病症或疾病,所述病症或疾病包括但不限于卒中、脑瘫、创伤性损伤和神经变性疾病。神经变性疾病的实例包括但不限于多发性硬化、亨廷顿病、阿尔茨海默病或帕金森病。

[0201] 另一个实施方案公开上文所述的式(I)的三萜衍生物,其用于治疗响应于HIF途径激活的病症或疾病,所述病症或疾病包括但不限于IBD,心肌缺血-再灌损伤,急性肺损伤,糖尿病性和慢性伤口,器官移植,急性肾损伤或动脉病。更优选地,所述病症或疾病选自IBD,心肌缺血-再灌损伤,急性肺损伤,器官移植,急性肾损伤或动脉病。

[0202] 本发明的一个实施方案还公开了上文所述的式(I)的化合物,其用于治疗响应于HIF途径激活的病症或疾病,其中所述响应于HIF途径激活的病症或疾病是代谢病理学。在优选的实施方案中,所述代谢病理学是糖尿病。在另一个优选的实施方案中,所述代谢病理学是脂质代谢病症。在另一个优选的实施方案中,所述代谢病理学是高脂血症。在另一个优选的实施方案中,所述代谢病理学是高甘油三酯血症。

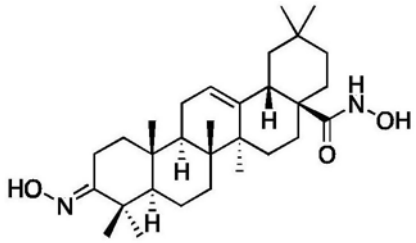
[0203] 本文公开的另一个实施方案是指式(I)的化合物或其选自(II),(III),(IV),(V),(VI),(VII),(VIII),(IX),(X),(XI),(XII),(XIII),(XIV)或(XV)的类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物。

[0204] (II)

[0205] 

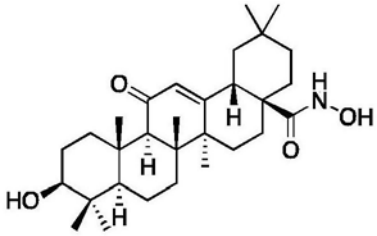
(3β) 3-羟基-*N*-羟基-齐墩果-12-烯-28-酰胺

[0206] (III)

[0207] 

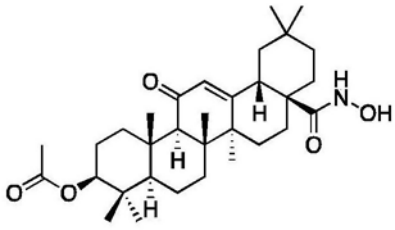
3-羟基亚胺基-*N*-羟基-齐墩果-12-烯-28-酰胺

[0208] (IV)

[0209] 

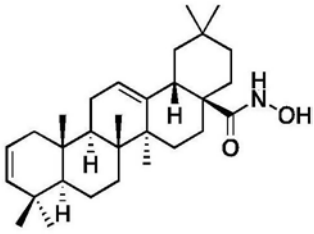
(3β) 3-羟基-11-氧代-齐墩果-12-烯-*N*-羟基-28-酰胺

[0210] (V)

[0211] 

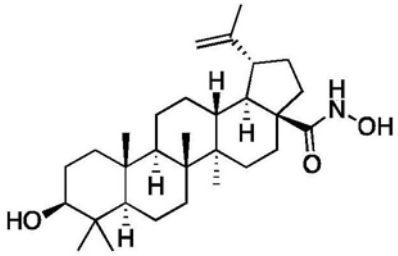
(3β) 3-乙酰氧基-11-氧代-齐墩果-12-烯-*N*-羟基-28-酰胺

[0212] (VI)

[0213] 

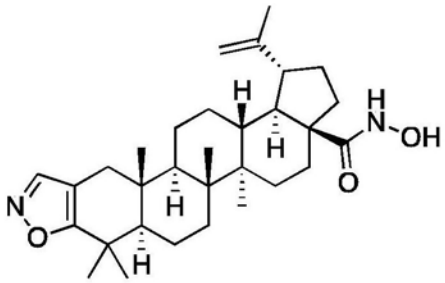
齐墩果-2,12-二烯-*N*-羟基-28-酰胺

[0214] (VII)

[0215] 

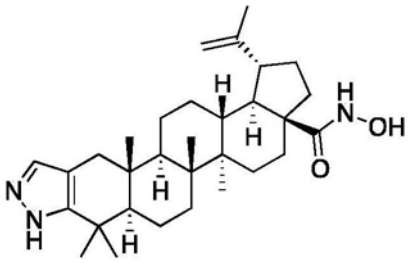
(3β) 3-羟基-*N*-羟基-羽扇-20(29)-烯-28-酰胺

[0216] (VIII)

[0217] 

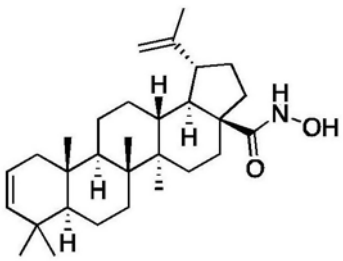
羽扇-2-烯并[2,3-d]异噁唑-*N*-羟基-28-酰胺

[0218] (IX)

[0219] 

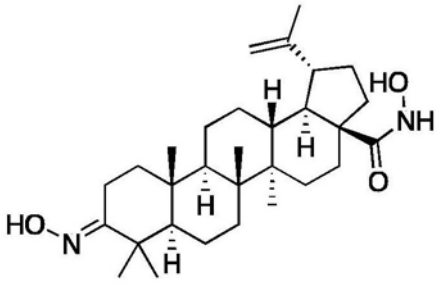
1'H-羽扇-20(29)-烯并[3,2-c]吡唑-*N*-羟基-28-酰胺

[0220] (X)

[0221] 

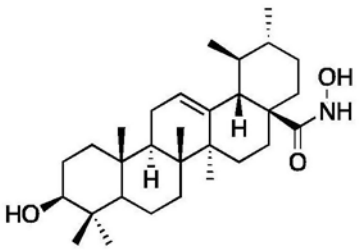
羽扇-2,20(29)-二烯-*N*-羟基-28-酰胺

[0222] (XI)

[0223] 

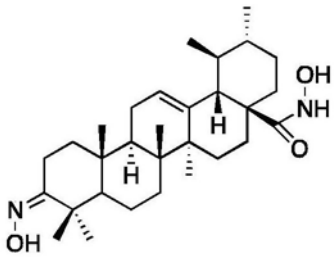
3-羟基亚胺基-*N*-羟基-羽扇-20(29)-烯-28-酰胺

[0224] (XII)

[0225] 

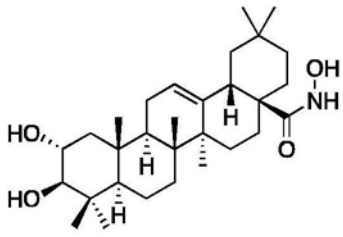
(3β) 3-羟基-*N*-羟基-乌苏-12-烯-28-酰胺

[0226] (XIII)

[0227] 

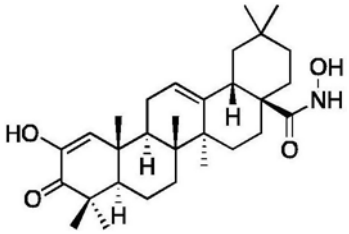
3 羟基亚胺基-*N*-羟基-乌苏-12-烯-28-酰胺

[0228] (XIV)

[0229] 

(2α,3β) 2,3-二羟基-*N*-羟基-齐墩果-12-烯-28-酰胺

[0230] (XV)

[0231] 

[0232] **2-羟基-3-氧代-齐墩果-1,12-二烯-*N*-羟基-28-酰胺**

[0233] 其用于治疗响应于HIF途径激活的病症或疾病。

[0234] 一个优选的实施方案是指式(I)的化合物或其选自(II),(III),(IV),(V),(VI),(VII),(VIII),(X),(XI),(XII),(XIII),(XIV)或(XV)的类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物,其用于治疗响应于HIF途径激活的病症或疾病。

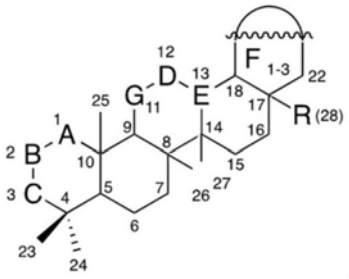
[0235] 上文提及的化合物II至XV或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物表现出下述能力:结合PHD2,稳定HIF-1α和HIF-2α蛋白,激活不同细胞类型中的HIF途径,诱导人内皮血管细胞中的血管发生,在体外和体内表现出神经保护活性,抗糖尿病活性和在体内降低脂质水平,以及在体内提高红细胞生成素血浆水平。所述式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物以选择性方式作用并且不诱导Nrf2激活、NF-κB抑制、STAT3抑制和TGR5激活,这些是它们天然的三萜系化合物前体的已知活性。

[0236] 如实施例1所示,从已知的天然三萜酸衍生物(桦木酸、齐墩果酸、熊果酸或山楂酸)或天然三萜酸本身起始,并且以通式(I)的化合物结束,使用常用的合成路线将位置28的酸基团转化成氧肟酸衍生物,其中,取决于式(I)的最终化合物的官能性,针对每种具体式(I)的化合物,进行其他另外的合成步骤。

[0237] 本发明的化合物III至VI可以从已知的天然齐墩果酸衍生物起始合成。本发明的化合物VIII至XI可以从天然桦木酸或其已知的衍生物起始合成。本发明的化合物XIV和XV可以从已知的天然山楂酸衍生物起始合成。

[0238] 实施例1还记载了化合物XVII和XVIII以及XIX,它们分别是化合物XV和XIV合成中的新型化合物中间体。

[0239] 本文公开的另一个实施方案是指药物组合物,其包含至少一种式(I)的三萜衍生物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物,

[0240] 

式(I)

[0241] 其中独立的,

[0242] -A-B选自碳-碳单键或碳-碳双键;

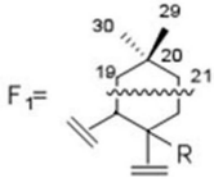
[0243] -B选自亚甲基(-CH_2-),烯次甲基(=CH-),羟基次甲基[-CH(OH)-],或羟基化烯碳[-C(OH)=];

[0244] -B-C选自碳-碳单键或碳-碳双键;或是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮;

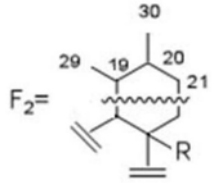
[0245] -C选自羟基次甲基[-CH(OH)-],酰氧基次甲基[-CH(OCOR')-],烯次甲基(=CH-),羰基[-C(=O)-],肟[-C(=N-OH)-]或腙[-C(=N-NH_2)-],其中R'是甲基;

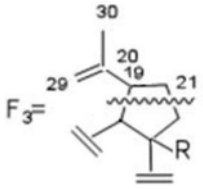
[0246] -D-E是碳-碳单键或双键;

[0247] -F选自F_1,F_2或F_3;



[0248]





[0249] -G选自亚甲基(-CH_2-)或羰基[-C(=O)-];并且

[0250] -R是氧肟酸基(-CONHOH);

[0251] 其用于治疗响应于HIF途径激活的病症或疾病。

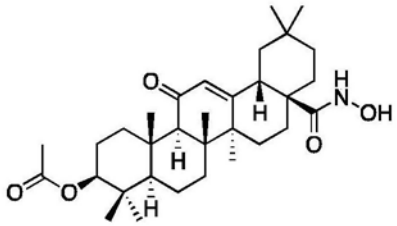
[0252] 在包含本文上文公开的式(I)的三萜衍生物的药物组合物的一个实施方案中,B-C可以是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮,并且其中所述杂环是五元环。

[0253] 在包含本文上文公开的式(I)的三萜衍生物的药物组合物的一个实施方案中,B-C可以是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮,并且其中所述杂环是包含两个氮原子的五元环。

[0254] 在包含本文上文公开的式(I)的三萜衍生物的药物组合物的一个实施方案中,B-C可以是包含一个或多个杂原子的杂环的一部分,其中至少一个所述杂原子是氮,并且其中所述杂环是包含一个氮和一个氧的五元环。

[0255] 在式(I)的三萜衍生物的一个实施方案中,当C是酰氧基次甲基[-CH(OCOR')-]时,式(I)的三萜衍生物是

[0256] (V)

[0257] 

(3β) 3-乙酰氧基-11-氧代-齐墩果-12-烯-*N*-羟基-28-酰胺；

[0258] 所述包含式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物的药物组合物表现出下述活性：结合PHD2，稳定HIF-1α和HIF-2α蛋白，激活不同细胞类型中的HIF途径，诱导人内皮血管细胞中的血管发生，在体外和体内表现出神经保护活性，抗糖尿病活性和在体内降低脂质水平，以及在体内提高红细胞生成素血浆水平。所述包含式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物的组合物以选择性方式作用，并且不诱导Nrf2激活、NF-κB抑制、STAT3抑制和TGR5激活，这些是它们天然的三萜系化合物前体的已知活性。

[0259] 本文公开的另一个实施方案是指包含至少一种上文所述的式(I)的三萜衍生物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物的药物组合物，其用于治疗响应于HIF途径激活的病症或疾病，所述病症或疾病包括但不限于卒中、脑瘫、创伤性损伤和神经变性疾病；或其用于治疗响应于HIF途径激活的病症或疾病，所述病症或疾病包括但不限于IBD，心肌缺血-再灌损伤，急性肺损伤，糖尿病性和慢性伤口，器官移植，急性肾损伤或动脉病。更优选地，所述病症或疾病选自IBD，心肌缺血-再灌损伤，急性肺损伤，器官移植，急性肾损伤或动脉病。

[0260] 本文公开的另一个实施方案是指包含本文上文公开的至少一种式(I)的三萜衍生物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物的药物组合物，其用于治疗响应于HIF途径激活的病症或疾病，其中所述响应于HIF途径激活的病症或疾病是代谢病理学。在优选的实施方案中，所述代谢病理学是糖尿病。在另一个优选的实施方案中，所述代谢病理学是脂质代谢病症。在另一个优选的实施方案中，所述代谢病理学是高脂血症。在另一个优选的实施方案中，所述代谢病理学是高甘油三酯血症。

[0261] 所述组合物可以进一步包含另一种活性成分，其在施用给人或动物时发挥治疗效果。

[0262] 典型的组合物包含本文上文所述的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物，其与药用赋形剂相关联，例如，所述药用赋形剂可以是载体或稀释剂。所述组合物可以是胶囊、小药囊、纸或其他容器的形式。在制备所述组合物时，可以使用用于制备药物组合物的常规技术。例如，上文公开的式(I)的化合物可以与载体混合，或通过载体稀释，或包封在载体中，其可以以安瓿瓶、胶囊、小药囊、纸或其他容器的形式存在。当载体作为稀释剂时，其可以是固体、半固体或液体材料，作为活性化合物的媒介物、赋形剂或介质。上文所述的式(I)的化合物可以吸附在粒状固体容器中，例如，在小囊袋中。适当的载体的一些实例是水、盐溶液、醇、聚乙二醇、聚

羟基乙氧基化的蓖麻油、花生油、橄榄油、乳糖、白陶土、蔗糖、环糊精、直链淀粉、硬脂酸镁、滑石、明胶、琼脂、果胶、阿拉伯胶、硬脂酸或纤维素的低级烷基醚、硅酸、脂肪酸、脂肪酸胺、脂肪酸单甘油酯和甘油二酯、季戊四醇脂肪酸酯、聚氧乙烯、羟甲基纤维素和聚乙烯吡咯烷酮。类似地，载体或稀释剂可以包括本领域已知的任何缓释物质，诸如单硬脂酸甘油酯或二硬脂酸甘油酯，其单独使用或与蜡混合。所述组合物还可以包含湿润剂，乳化剂和混悬剂，防腐剂，甜味剂或调味剂。可以配制本发明的组合物，从而在通过使用本领域中公知的步骤施用给患者后提供本文公开的式(I)的化合物的快速、持续或延迟的释放。

[0263] 可以将药物组合物灭菌，并且如果需要，与辅助剂、乳化剂、影响渗透压的盐、缓冲剂和/或着色物质等混合，其不与上文公开的化合物有害地反应。

[0264] 本文公开的一个优选的实施方案涉及施用途径，其可以是有效转运上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物至适当的或所需的作用位点的任何途径，如口服、经鼻、局部、经肺、经皮或肠胃外，例如直肠、皮下、静脉内、尿道内、肌肉内、鼻内、眼用溶液或软膏。

[0265] 为了鼻施用，所述组合物可以含有溶解或悬浮在液体载体，特别是水性载体中的上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物，用于气溶胶应用。载体可以含有添加剂如增溶剂，例如丙二醇，表面活性剂，吸收促进剂如卵磷脂(磷脂酰胆碱)，或环糊精，或防腐剂如对羟苯甲酸酯类(parabens)。

[0266] 为了制备局部制剂，将上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物置于本领域中已知的皮肤媒介物中。要施用的上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物的量和局部制剂中化合物的浓度取决于媒介物、选择的递送系统或装置、患者的临床状况、副作用和制剂中化合物的稳定性。因此，医生使用含有适当浓度的上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物的适当的制剂，并且选择要施用的制剂的量，这取决于讨论的患者或类似的患者的临床体验。

[0267] 为了眼用，将上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物配制为适于在眼中使用的溶液、悬浮液和软膏。浓度通常如上文对于局部制剂讨论的。

[0268] 为了口服施用，可以制备固体或液体单位剂型。为了制备固体组合物如片剂，将上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物与作为药物稀释剂或载体的常规成分如滑石、硬脂酸镁、磷酸二钙、硅酸镁铝、硫酸钙、淀粉、乳糖、阿拉伯胶、甲基纤维素，和功能类似的材料混合成制剂。

[0269] 通过将上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物与惰性药物稀释剂混合并且将混合物装入适当尺寸的硬明胶胶囊中来制备胶囊。通过将式(I)的化合物与可接受的植物油、轻液体矿脂或其他惰性油的浆液机械封装入胶囊制备软明胶胶囊。可以制备用于口服施用的液体单位剂型如糖浆、酏剂和混悬剂。水溶形式可以与糖、芳香调味剂和防腐剂一起溶解在水性媒

介物中以形成糖浆。通过使用水醇的(hydroalcoholic)(例如,乙醇)媒介物与合适的甜味剂如糖和糊精,连同芳香调味剂制备酏剂。可以利用水性媒介物,在悬浮剂如阿拉伯胶、黄芪胶、甲基纤维素等的帮助下制备混悬剂。

[0270] 用于肠胃外使用的适当的组合物对于普通技术人员来说是明显的,如使用合适的可注射溶液或混悬液。无菌组合物适于各种局部或肠胃外途径,包括皮内、肌肉内、血管内和皮下。

[0271] 除了上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物,取决于组合物和所需递送模式,所述组合物可以包括药用、非毒性载体或稀释剂,其包括通常用于形成用于动物或人施用的药物组合物的媒介物。选择稀释剂从而不会不适当地影响组合的生物活性。

[0272] 特别用于可注射制剂的这样的稀释剂的实例是水、各种盐水、有机或无机盐溶液、Ringer's溶液、葡萄糖溶液和Hank's溶液。此外,药物组合物或制剂可以包括添加剂如其他载体;辅剂;或无毒、非治疗性、非免疫原性稳定剂等。

[0273] 此外,赋形剂可以包括在公开的组合物中。实例包括共溶剂、表面活性剂、油、保湿剂、润滑剂、防腐剂、稳定剂和抗氧化剂。可以使用任何药理学可接受的缓冲液,例如tris或磷酸盐缓冲液。稀释剂、添加剂和赋形剂的有效量是有效获得关于溶解度、生物活性等的药用制剂的那些量。

[0274] 上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物可以并入微球中。上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物可以加载入白蛋白微球中,由此可能将该微球恢复在干粉中用于鼻内施用。其他适于制备微球的材料包括琼脂、藻酸盐、壳聚糖、淀粉、羟基乙基淀粉、白蛋白、琼脂糖、葡聚糖、透明质酸、明胶、胶原蛋白和酪蛋白。微球可以通过本领域技术人员已知的各种方法制造,如喷雾干燥方法或乳化方法。

[0275] 例如,可以通过将磷酸缓冲液中的兔血清白蛋白加入橄榄油中并搅拌以产生油包水乳状液来制备白蛋白微球。然后将戊二醛溶液加入至乳状液中,并将乳状液搅拌以交联白蛋白。然后可以通过离心将微球分离,去除油并且例如用石油醚洗涤球,接着用乙醇洗涤。最后,可以将微球过筛并收集并通过过滤干燥。

[0276] 可以通过将温的淀粉水溶液,例如马铃薯淀粉的水溶液加入至热的聚乙二醇的水溶液,并搅拌以形成乳状液来制备淀粉微球。当形成两相(淀粉溶液作为内部相)体系时,然后在连续搅拌下将混合物冷却至室温,此时内部相转变为凝胶颗粒。然后在室温将这些颗粒过滤掉,并在溶剂如乙醇中成浆,这之后再次将颗粒滤去并在空气中放置至干燥。微球可以通过公知的交联步骤如热处理或通过使用化学交联剂硬化。合适的试剂包括二醛,包括乙二醛、丙二醛、丁二醛、己二醛、戊二醛和苯二醛,二酮如丁二酮,表氯醇,多磷酸盐和硼酸盐。二醛用于通过与氨基相互作用来交联蛋白如白蛋白,并且二酮与氨基形成希夫碱。表氯醇利用亲核试剂如氨基或羟基活化化合物以得到环氧化物衍生物。

[0277] 本发明的另一优选的实施方案是上文所述的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物以及包含所述化合物的组合物的剂量方案。术语"单位剂型"是指适合作为用于受试者,例如,哺乳动物受试

者，例如人、狗、猫和啮齿类动物的单一剂量的物理上分离的单位，各个单位含有与所要求的药物稀释剂、载体或媒介物关联的预定量的活性物质，所述活性物质的预定量计算为产生所需药效。用于本发明的单位剂型的规格(specifications)受控于并且依赖于：(a)上文公开的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物的独特特性和要获得的特别效果和(b)将所述式(I)的化合物配料用在人和动物中的领域中固有的限制。单位剂型的实例是片剂、胶囊、丸剂、粉末包、薄饼(wafer)、栓剂、颗粒、扁囊剂、茶匙、汤匙、滴剂(dropperfuls)、安瓿、小瓶、计量释放的气溶胶、前述任一项的隔离的多次量、和本文中所述的其他形式。本文公开的组合物可以包括在试剂盒中，所述试剂盒可以含有一种以上单位剂型的组合物和用于治疗本文所述的一种以上疾病的使用说明。

[0278] 缓慢或延长释放递送系统，包括很多生物聚合物(基于生物的系统)、使用脂质体的系统、胶体、树脂和其他多聚递送系统或隔室化的储库中的任一种，可以与本文中所述的组合物一起使用以提供治疗性化合物的连续或长期来源。这样的缓慢释放系统可用于经局部、眼内、口服和肠胃外途径递送的制剂。

[0279] 所述组合物中包含有效量的上文所述的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物。根据本发明使用的化合物的剂量根据化合物和治疗的状况例如接受者患者的年龄、体重和临床状况而改变。其他因素包括：施用途径、患者、患者的医疗史、疾病过程的严重度和具体的化合物的效力。剂量应该足以缓解治疗的疾病的症状或征兆，而不对患者产生不可接受的毒性。通常，有效量的化合物是提供由临床医师或其他有资质的观察者注意的主观的症状减轻或客观的可鉴定的改善的量。

[0280] 本文公开的一个实施方案涉及使用式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物治疗响应于HIF途径激活的病症或疾病的方法。

[0281] 所述式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物表现出下述能力：结合PHD2，稳定HIF-1α和HIF-2α蛋白，激活不同细胞类型中的HIF途径，诱导人内皮血管细胞中的血管发生，在体外和体内表现出神经保护活性，抗糖尿病活性和在体内降低脂质水平，以及在体内提高红细胞生成素血浆水平。所述式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物以选择性方式作用并且不诱导Nrf2激活、NF-κB抑制、STAT3抑制和TGR5激活，这些是它们天然的三萜系化合物前体的已知活性。

[0282] 本文公开的另一个实施方案涉及使用式(I)的化合物治疗响应于HIF途径激活的疾病的方法，所述疾病诸如IBD，心肌缺血-再灌损伤，急性肺损伤，糖尿病性和慢性伤口，器官移植，急性肾损伤或动脉病。在优选的实施方案中，所述疾病选自IBD，心肌缺血-再灌损伤，急性肺损伤，器官移植，急性肾损伤或动脉病。

[0283] 本文公开的另一个实施方案涉及使用式(I)的化合物治疗响应于HIF途径激活的病症或疾病的方法，所述病症或疾病选自卒中、脑瘫、创伤性损伤或神经变性疾病。

[0284] 本文公开的一个实施方案涉及治疗响应于HIF途径激活的病症或疾病的方法，所述病症或疾病选自IBD，心肌缺血-再灌损伤，急性肺损伤，糖尿病性和慢性伤口，器官移植，

急性肾损伤或动脉病，所述方法包括向需要所述治疗的个体施用有效量的上文所述的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物。在优选的实施方案中，所述疾病选自IBD，心肌缺血-再灌损伤，急性肺损伤，器官移植，急性肾损伤或动脉病。

[0285] 本文公开的另一个实施方案涉及治疗响应于HIF途径激活的病症或疾病的方法，所述所述包括向需要所述治疗的个体施用有效量的上文所述的式(I)的化合物或其类似物、衍生物、互变异构体形式、异构体、立体异构体、多晶型物、药用盐或药用溶剂化物。

[0286] 在本文公开的一个实施方案中，响应于HIF途径激活的病症或疾病包括但不限于卒中，脑瘫，创伤性损伤和神经变性疾病。神经变性疾病的实例包括但不限于阿尔茨海默病，帕金森病，亨廷顿病或多发性硬化。

[0287] 附图简述

[0288] 图1.与PHD2结合的桦木酸(图1A)和化合物VII(图1B)的高评分构象(PDB 4BQW)。

[0289] 图2.在HaCaT-EPO Luc角质形成细胞中的HIF-1α反式激活测定。

[0290] 在HaCaT-EPO-Luc细胞中DFX和化合物II至XV的低氧模拟作用。测试的化合物的浓度(μM)显示在x-轴上，并且HIF-1α激活的百分数显示在y-轴上。该图显示DFX相对于化合物(图2A)II，III，IV，V，VI，VII，XI，(图2B)VIII，IX，X，XII，XIII，XIV和XV对EPO-luc活性的作用，将由DFX(150μM)介导的诱导视为相对于未处理细胞的100％激活。

[0291] 图3.在少突胶质细胞中的HIF-1α稳定化。

[0292] 用150μDFX或10μM齐墩果酸(OA)，化合物II，III，IV，V，VI(图3A)，桦木酸(BA)，化合物VII，VIII，IX，X，XI(图3B)，熊果酸(UA)，山楂酸(MA)，化合物XII，XIII，XIV和XV(图3C)刺激人少突胶质细胞MO13.3细胞3小时，以通过蛋白质印迹确定HIF-1α和β-肌动蛋白的表达。

[0293] 图4.在293T细胞中的HIF-1α稳定化。

[0294] 在3小时期间用增加浓度的化合物VII或用DFX(150μM)刺激人胚肾293细胞。通过蛋白质印迹确定蛋白HIF-1α、PHD1、PHD2和β-肌动蛋白的稳定状态水平。

[0295] 图5.在hIPC中的HIF-2α稳定化。

[0296] 在3小时期间用增加浓度的化合物VII或用DFX(150μM)刺激人胰岛来源的前体细胞(hIPC)，并且通过蛋白质印迹确定HIF-2α、PHD3和β-肌动蛋白的表达。

[0297] 图6.化合物VII诱导血管生成。

[0298] 在与原代成纤维细胞共同培养并分别用化合物VII(1μM)、rhFGF(10ng/ml)和VEGFA(10ng/ml)刺激7天的绿色荧光人内皮血管细胞(HUVEC)中，测量内皮细胞管形成，其作为血管生成模型。值表示平均值±SEM(n＝3)。

[0299] 图7.在体内化合物VII对红细胞生成素(EPO)的影响。

[0300] 使用小鼠EPO ELISA试剂盒，测量腹膜内施用了化合物VII(30mg/kg或60mg/kg)或桦木酸(BA)(60mg/kg)的C57BL/6雄性小鼠血浆中的EPO。对照组没有接受任何处理。数据表示为平均值±SEM(n＝3)。

[0301] 图8.在Q7和Q11纹状体细胞中，化合物VII消除3-NP毒性。将Q7和Q111纹状体细胞用增加浓度的化合物VII预处理6小时，然后暴露于3-NP(10mM)另外的24小时，之后利用YOYO-1染色来研究细胞死亡。使用IncuCyte HD软件确定细胞死亡，并且将在Q111细胞中仅

用3-NP处理视为100％的细胞死亡。

[0302] 图9.3NP中毒后的行为评分。

[0303] 使3-硝基丙酸(3-NP)中毒的小鼠进行行为测试,以确定在用化合物VII(30mg/kg)和桦木酸(BA)(30mg/kg)处理后相对于未中毒的小鼠对照它们的神经学状况。基于严重性,对后肢抱握、运动活动、后肢张力障碍和躯干张力障碍按0-2分级:评分0通常表示正常功能,2表示严重影响。值表示为每组6只动物的平均值±SEM。

[0304] 图10.化合物VII对神经元损失的影响。

[0305] 用3-硝基丙酸(3-NP)中毒的小鼠用于确定纹状体中的变性,包括对照(未中毒的小鼠),3NP,3NP+BA和3NP+化合物VII)。小鼠纹状体中Nissl-阳性细胞的定量。显示了神经元的总平均数(放大100倍)。值表示为每组6只动物的平均值±SEM。数据进行单向方差分析,然后进行Student-Newman-Keuls检验。当比较对照组与3NP和对照组时,***P<0.001。当比较3NP组与3NP+化合物VII组时,#P<0.05。

[0306] 图11.化合物VII对3NP诱导的小胶质细胞增生($Iba1^+$)和星形胶质细胞增生($GFAP^+$)的作用

[0307] 用3-硝基丙酸(3-NP)中毒的小鼠用于确定小胶质细胞激活和小胶质细胞增生,包括对照(未中毒的小鼠),3NP,3NP+BA和3NP+化合物VII)。通过对来自不同组小鼠的脑切片免疫染色来确定Iba-1和神经胶质原纤维酸性蛋白(GFAP)表达,并且使用Image J软件进行不同标记的定量。显示了小胶质细胞($Iba1^+$)和星形胶质细胞($GFAP^+$)的总平均数。

[0308] 图12.化合物VII减少纹状体中炎性标记mRNA的表达。

[0309] 与3NP+媒介物小鼠相比,在3NP+化合物VII(30mg/kg)处理的小鼠中,包括COX-2(图12D)、IL-1β(图12B)、IL-6(图12A)和iNOS(图12C)的炎性标记的基因表达被下调。桦木酸(BA)处理(30mg/kg)也抑制炎性标记的表达。利用$2^{-\Delta\Delta Ct}$法计算表达水平。值表示为每组6只动物的平均值±SEM。

[0310] 图13.化合物VII对进行高脂肪饮食的小鼠(HFD动物)的体重、脂肪量和肥胖的影响。

[0311] 将小鼠用HFD喂养13周,并每日用化合物VII(30mg/Kg)处理21天,每3-4天监测对体重的影响(图13A)。％瘦肉量(图13B)计算为没有脂肪部分的总体重的比例。％脂肪量(图13C)计算为脂肪与总体重的比例。在第15周计算脂肪量和肥胖百分数。通过磁共振成像(MRI)评估身体组成。值表示为每组10只动物的平均值±SEM。

[0312] 图14.化合物VII改善HFD动物中的葡萄糖耐量(GTT)。

[0313] 3周媒介物(对照,CD)或化合物VII施用(30mg/Kg)后HFD-喂养的小鼠的GTT。将与每只动物相对应的在0,15,30,45,60,90和120分钟时间点之间的梯形面积和求和,以得到曲线下面积(AUC)。相对面积值表示为相对于媒介物组的平均AUC(将其定义为100％)的百分数。值表示为平均值±S.E.M(n＝6)。

[0314] 图15.化合物VII预防HFD动物中的高甘油三酯血症。

[0315] 3周媒介物(对照,CD)或化合物VII施用(30mg/Kg)后对照和HFD-喂养的小鼠中的血浆甘油三酯水平。值表示为平均值±S.E.M(n＝6)。

实施例

[0316] 下文所述的本发明的实施例目的在于举例说明其优选的实施方案,而不是限制其保护范围。

[0317] 实施例1.合成式(I)的三萜系化合物衍生物和比较化合物XX。

[0318] 式(I)的N-羟基-三萜-28-酰胺衍生物的一般合成(氧肟酸形成):

[0319] 向在无水二氯甲烷(DCM)中的三萜酸前体(1eq/mol)的冰冷溶液中,逐滴加入草酰氯(6eq/mol),并将混合物在40℃加热1,5小时。在真空中去除溶剂,将残余物溶解在无水吡啶或N,N-二异丙基乙胺(DiPEA)中,并加入氯化羟铵(6eq/mol)。将反应物在40℃加热3小时,用2N H_2SO_4溶液猝灭,并且用EtOAc萃取。有机相用盐水洗涤,用Na_2SO_4干燥并在真空中挥发。在硅胶上纯化粗制化合物。

[0320] 可能的话,利用其CAS编号标记已知的化合物。

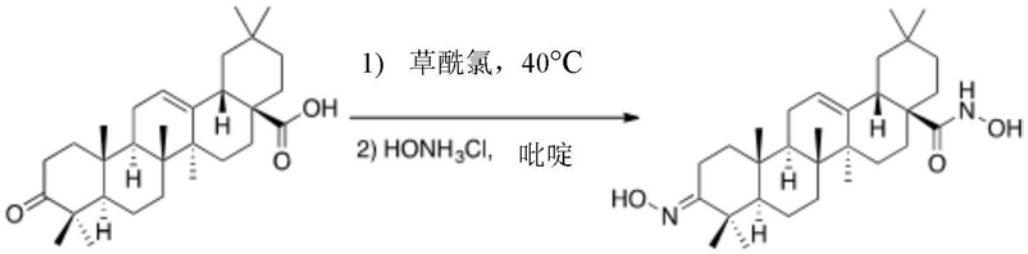
[0321] (3β)3-羟基-N-羟基-齐墩果-12-烯-28-酰胺(化合物II)

[0322] CAS编号:1854922-22-7

[0323] 灰白色固体(60%)。^{1}H NMR(300MHz,$CDCl_3$):d:^{1}H NMR(300MHz,$CDCl_3$):d=5.41(brt,1H,H-12),3.19(dd,J=10.0,4.89Hz,1H),2.44(d,J=10.8Hz,1H),2.05-1.86(m,3H),1.16(s,3H),0.98(s,3H),0.92(s,3H),0.90(s,3H),0.88(s,3H),0.81(s,3H),0.78(s,3H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$):d=176.7,144.9,123.9,78.9,55.2,47.6,46.4,45.5,42.0,40.8,39.5,38.8,38.6,37.0,34.0,32.9,32.3,32.0,30.7,28.1,27.3,27.2,25.84,23.7,23.6,23.5,18.3,16.7,15.6,15.4。

[0324] 3-羟基亚胺基-N-羟基-齐墩果-12-烯-28-酰胺(化合物III)

[0325] 按照本文上文所述的一般合成方案(氧肟酸形成),从已知的前体CAS编号17990-42-0起始,得到化合物III:

[0326] 

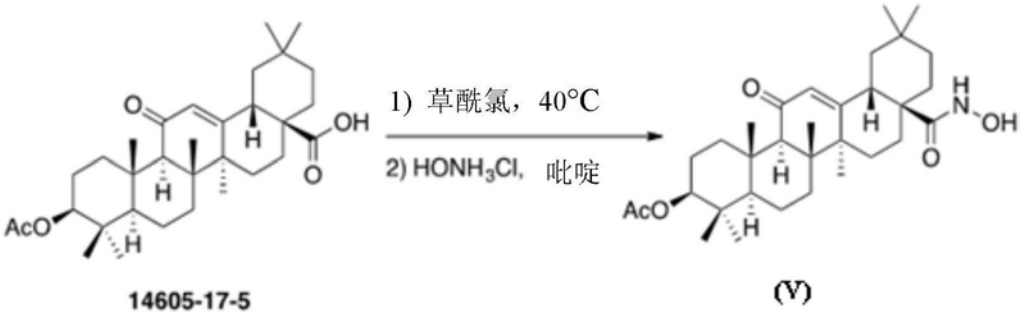
17990-42-0 (III)

[0327] 在相同的一般合成路径中进行化合物III位置C特有的肟形成以及氧肟酸形成。

[0328] 灰白色固体(70%)。^{1}H NMR(300MHz,$CDCl_3$):d=5.43(brt,1H),3.07(bdt,J=14.9,1H),2.46(m,1H),1.98(m,3H),1.13(s,10H),1.07(s,6H),1.05(s,3H),0.86(s,6H),0.82(s,3H)(仅报告了容易的峰);^{13}CNMR(75MHz,$CDCl_3$)d=176.7,167.5,144.9,123.8,55.7,47.1,46.2,45.5,42.0,40.8,40.3,39.4,38.4,37.0,33.9,32.9,31.9,30.7,29.7,29.2,27.2,25.7,25.5,23.7,23.5,23.4,19.0,17.3,16.7,14.9。

[0329] (3β)3-乙酰氧基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺(化合物V)

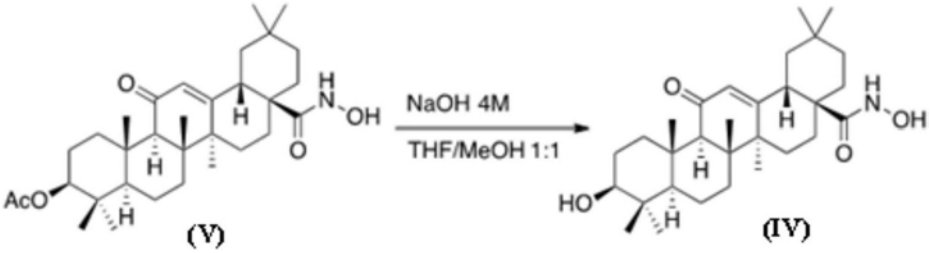
[0330] 按照本文上文所述的一般合成方案(氧肟酸形成),从已知的前体CAS编号14605-17-5得到化合物V。

[0331] 

[0332] 浅黄色固体(75％)。^{1}H NMR(300MHz,$CDCl_3$):d＝5.65(s,1H),4.47(dd,J＝4.8J＝11.0Hz,1H),3.31(m,1H),2.76(t,J＝12.5Hz,2H),2.32(s,1H),2.00(s,3H),1.12(s,3H),0.91(s,3H),0.88(s,6H),0.82(s,9H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$)d＝200.5,174.4,171.2,168.4,127.8,80.7,61.9,55.0,45.3,44.8,43.6,43.0,40.7,38.7,38.0,37.1,33.8,32.8,32.6,32.1,30.7,28.1,27.3,23.6,23.5,23.4,23.1,21.3,19.0,17.3,16.7,16.3。

[0333] (3β)3-羟基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺(化合物IV)

[0334] 为了得到化合物IV,按下述进行化合物V的去乙酰化：

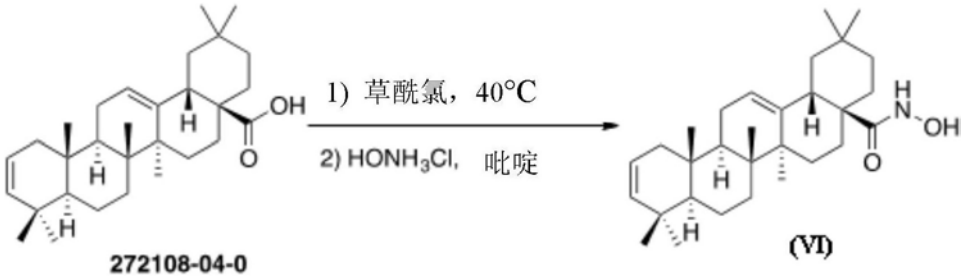
[0335] 

[0336] 向化合物V,(3β)3-乙酰氧基-11-氧代-齐墩果-12-烯-N-羟基-28-酰胺(1eq/mol)在THF/MeOH 1:1中的溶液中,加入NaOH 4N(50eq/mol)。将混合物在40℃加热过夜,用2N H_2SO_4溶液猝灭,并用EtOAc萃取。将有机相用盐水洗涤,用Na_2SO_4干燥,并在真空中挥发。在硅胶上纯化粗制化合物。

[0337] 浅黄色固体(65％)。^{1}H NMR(300MHz,$CDCl_3$):d＝5.67(s,1H),3.20(t,J＝6.1Hz,1H),2.74(d,J＝12.2Hz,2H),2.32(s,1H),2.10-2.02(m,1H),1.18(s,3H),0.96(s,3H),0.94(s,3H),0.90(s,9H),0.77(s,3H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$)d＝200.2,175.8,167.9,128.0,78.8,62.1,55.0,45.2,44.7,43.6,40.9,39.2,37.3,33.7,32.8,32.7,32.1,30.7,29.7,28.1,27.4,27.3,23.7,23.4,23.3,19.0,17.5,16.2,15.6,14.2。

[0338] 齐墩果-2,12-二烯-N-羟基-28-酰胺(化合物VI)

[0339] 按照本文上文所述的一般合成方案(氧肟酸形成),从已知的前体CAS编号272108-04-0起始,得到化合物VI：

[0340] 

272108-04-0 (VI)

[0341] 黄色油(48%)。^{1}H NMR(300MHz,$CDCl_3$):d＝5.43-5.32(m,3H),2.44(d,J＝11.3Hz,1H),1.14(s,3H),0,97(s,6H),0,87(s,12H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$)d＝176.4,144.5,138.0,124.1,121.3,51.9,46.4,46.1,45.5,42.1,41.0,40.7,39.5,36.1,34.5,34.0,33.0,31.9,31.8,31.6,30.7,27.2,25.9,25.7,23.8,23.5,22.9,19.6,16.3,15.6。

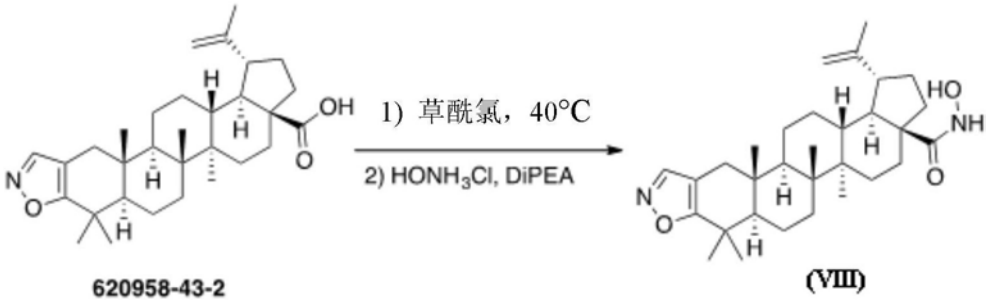
[0342] (3β)3-羟基-N-羟基-羽扇-20(29)-烯-28-酰胺(化合物VII)

[0343] CAS编号:1822375-07-4

[0344] 灰白色固体(55%)。^{1}H NMR(300MHz,$CDCl_3$):d:4.73(s,1H),4.60(s,1H),3.20-3.15(m,1H),3.05(ddd,J＝11.4,6,4.49Hz,1H),1.67(s,3H),0.95(s,6H),0.92(s,3H),0.80(s,3H),0.74(s,3H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$):d:175.2,150.9,108.5,78.4,55.7,54.3,50.8,50.7,46.7,42.3,40.7,38.7,38.2,37.8,37.1,36.9,34.3,32.6,30.7,29.3,27.8,26.9,25.5,20.8,19.2,18.2,16.0,15.9,15.2,14.5。

[0345] 羽扇-2-烯并[2,3-d]异噁唑-N-羟基-28-酰胺(化合物VIII)

[0346] 按照本文前文所述的一般合成方案(氧肟酸形成),从已知的前体CAS编号620958-43-2起始,得到化合物VIII:

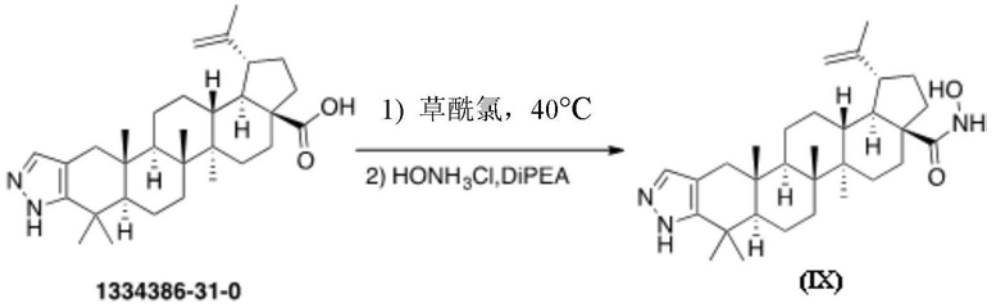
[0347] 

620958-43-2 (VIII)

[0348] 白色固体。^{1}H NMR(300MHz,CD_3OD):d＝10.36(s,1H,NH),8.32(s,1H,OH),8.26(s,1H),4.67(s,1H),4.55(s,1H),3.00(t,J＝9.3Hz,1H),2.61(t,J＝12.0Hz,1H),1.64(s,3H),1.40(s,3H),1.22(s,3H),1.11(s,3H),0.93(s,3H),0.74(s,3H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$)d＝172.6,151.3,151.0,109.9,109.4,54.0,53.3,50.6,49.0,48.9,46.7,42.4,38.9,37.3,35.6,34.8,33.4,32.6,30.9,29.0,25.7,21.7,19.5,18.7,16.4,16.2,14.8。

[0349] 1'H-羽扇-20(29)-烯并[3,2-c]吡唑-N-羟基-28-酰胺(化合物IX)

[0350] 按照本文前文所述的一般合成方案(氧肟酸形成),从已知的前体CAS编号1334386-31-0起始,得到化合物IX:

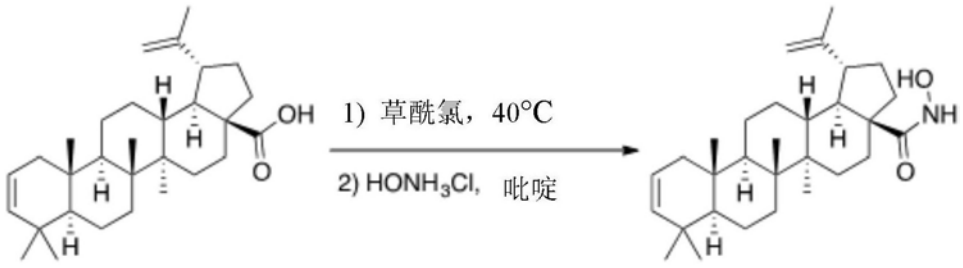
[0351] 

1334386-31-0 (IX)

[0352] 浅黄色固体。^{1}H NMR(300MHz，$(CD_3)_2CO$)：d＝7.17(s,1H)，4.72(s,1H)，4.58(s,1H)，3.20-3.13(m,1H)，2.69-2.61(m,1H)，1.69(s,3H)，1.28(s,3H)，1.18(s,3H)，1.02(s,3H)，0.98(s,3H)，0.80(s,3H)(仅报告了容易的峰)；^{13}C NMR(75MHz，$(CD_3)_2CO$)d＝172.4，151.0，149.1，132.8，111.9，109.0，59.7，50.0，53.7，50.5，49.2，46.8，42.2，40.7，38.6，37.9，37.7，36.6，33.5，33.4，32.3，30.8，30.6，25.7，23.3，21.4，19.1，18.7，15.6，14.2，13.7。

[0353] 羽扇-2,20(29)-二烯-N-羟基-28-酰胺(化合物X)

[0354] 按照本文前文所述的一般合成方案(氧肟酸形成)，从已知的前体CAS编号173106-19-9起始，得到化合物X：

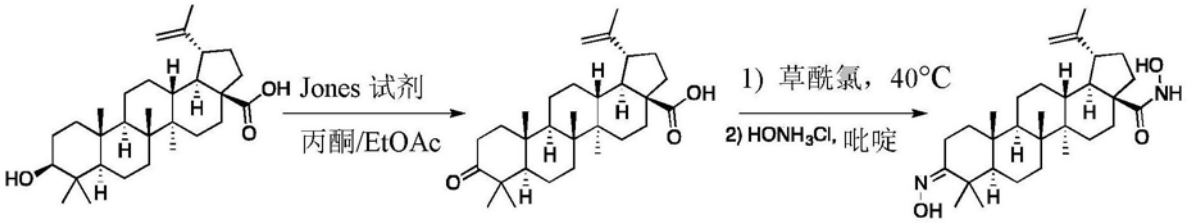
[0355] 

173106-19-9 (X)

[0356] 浅黄色固体(54％)^{1}H NMR(300MHz，$CDCl_3$)：d＝5.38-5.28(m,2H)，4.69(s,1H)，4.56(s,1H)，3.01(t,J＝10.7Hz,1H)，2.37(t,J＝12.1Hz,1H)，1.63(s,3H)，1.21(s,3H)，0,93(s,3H)，0,89(s,3H)，0,82(s,3H)，0,81(s,3H)(仅报告了容易的峰)；^{13}C NMR(75MHz，$CDCl_3$)d＝175.0，150.5，137.9，121.6，109.65，54.3，52.1，50.4，49.2，42.3，40.8，38.4，37.9，36.4，34.6，33.5，32.8，31.7，30.9，30.8，29.7，29.3，25.6，22.6，19.5，16.4，15.8，14.6，14.5，14.3。

[0357] 3-羟基亚胺基-N-羟基-羽扇-20(29)-烯-28-酰胺-步骤(b)-(化合物XI)

[0358] 在上文所述的一般合成方案(氧肟酸形成)之前，按下述进行桦木酸C-3位置羟基向羰基的转化：

[0359] 

(XVI) (XI)

[0360] 向桦木酸(1gr,2.19mmol)在丙酮/EtOAc 5:5(10mL)中的溶液中,加入Jones试剂,直到起始物质(通过TLC控制)消失。将反应物用盐水洗涤,并用EtOAc萃取。有机相用Na_2SO_4干燥并在真空中挥发,在硅胶(PE/EtOAc 9:1)上纯化粗制物,得到灰白色固体3-氧代-羽扇-20(29)-烯-28-酸(化合物XVI;CAS编号:4481-62-3)(89%)。

[0361] 然后按照上文所述的一般合成方案,使用化合物XVI进行氧肟酸形成,以得到化合物XI。

[0362] 灰白色固体(65%)。^{1}H NMR(300MHz,$CDCl_3$):d=4.73(s,1H),4.61(t,J=6.1Hz,1H),3.04-3.00(m,2H),2.32(s,1H),1.68(s,3H),1.24(s,6H),1.22(s,3H),1.12(s,3H),0.97(s,3H),0.96(s,3H),0.91(s,3H)(仅报告了容易的峰);177.3,167.3,150.5,109.6,55.6,55.0,50.3,50.2,46.8,42.5,40.8,40.2,38.7,38.2,37.9,37.2,34.0,33.3,30.8,29.4,27.4,25.6,22.9,21.5,21.2,19.4,19.1,16.1,15.8,14.6。

[0363] (3β)3-羟基-N-羟基-乌苏-12-烯-28-酰胺(化合物XII)

[0364] CAS编号:915415-61-1

[0365] 灰白色固体(55%)。^{1}H NMR(300MHz,$CDCl_3$):d=5.34(brt,1H,H-12),3.18(dd,J=9.5,3.9Hz,1H),2.09-1.92(m,3H),1.06(s,3H),0.94(s,3H),0.90(d,J=3.2Hz,3H),0.87(d,J=7.8Hz,3H),0.80(s,3H),0.78(s,3H),0.75(s,3H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$):d=176.8,140.1,126.6,126.6,79.0,55.1,52.2,47.5,42.4,39.5,39.5,39.0,38.8,36.9,28.1,25.7,23.3,21.2,17.2,16.7,15.7,15.5。

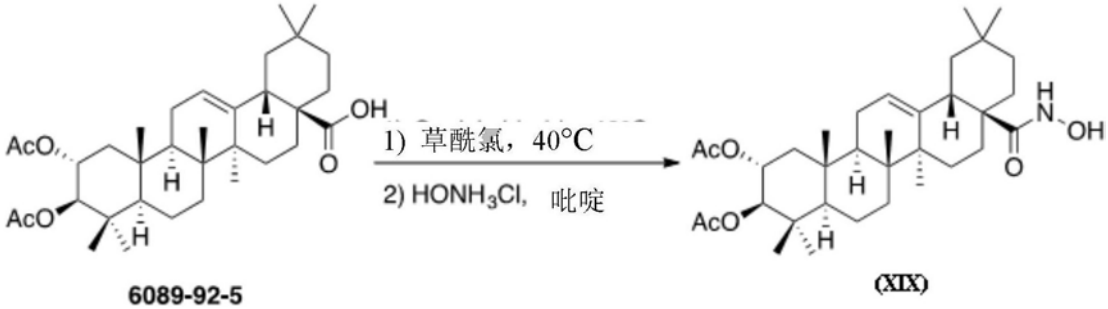
[0366] 3羟基亚胺基-N-羟基-乌苏-12-烯-28-酰胺(化合物XIII)

[0367] 专利CN102180939中公开的化合物。所述化合物通过进行与化合物XI类似的合成由熊果酸制备,其中在第一步,使用Jones试剂进行从C-3羟基到羰基的转化,然后按照上文所述的一般合成方案进行氧肟酸形成,以得到化合物XIII。

[0368] 浅黄色固体(70%)。^{1}H NMR(300MHz,$CDCl_3$):d=5.40(brt,1H),3.07(bdt,J=15.6,1H),2.12(m,1H),1.24(s,3H),1.15(s,3H),1.08(s,3H),1.06(s,3H),1.03(s,3H),0.94(s,3H),0.81(s,3H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$)d=177.3,167.7,140.6,126.5,55.7,52.1,47.0,42.5,40.2,39.6,39.4,39.0,38.5,37.0,36.7,32.2,30.6,29.7,27.7,27.4,24.8,23.5,23.4,23.3,21.1,19.0,17.3,17.2,16.8,15.1。

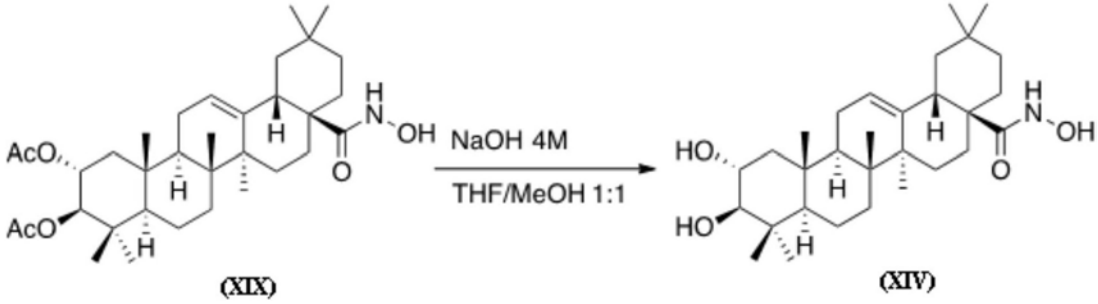
[0369] (2α,3β)2,3-二羟基-N-羟基-齐墩果-12-烯-28-酰胺(化合物XIV)

[0370] 按照本文前文所述的一般合成方案(氧肟酸形成),从已知的前体CAS编号:6089-92-5起始,得到化合物XIV:

[0371] 

6089-92-5 (XIX)

[0372] 将化合物XIX去乙酰化,得到化合物XIV:

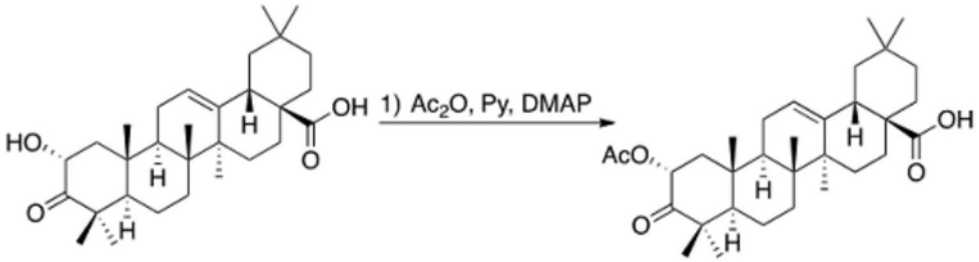
[0373] 

(XIX) (XIV)

[0374] 灰白色固体(45%)。^{1}H NMR(300MHz,$CDCl_3$):d＝5.44(brt,1H),3.72-3.61(m,1H),2.99(d,J＝9.5Hz,1H),2.45(d,J＝12.2Hz,1H),1.15(s,3H),1.02(s,3H),0.98(s,3H),0.90(s,3H),0.87(s,3H),0.82(s,3H),0.78(s,3H)(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$):d＝175.9,144.0,123.6,83.7,68.2,55.0,47.3,46.2,46.1,45.6,41.2,41.0,39.0,38.7,37.8,33.6,32.6,32.4,32.3,31.4,28.5,27.4,27.3,23.2,23.1,22.8,18.6,16.3,16.5,16.1。

[0375] 2-羟基-3-氧代-齐墩果-1,12-二烯-N-羟基-28-酰胺(化合物XV)

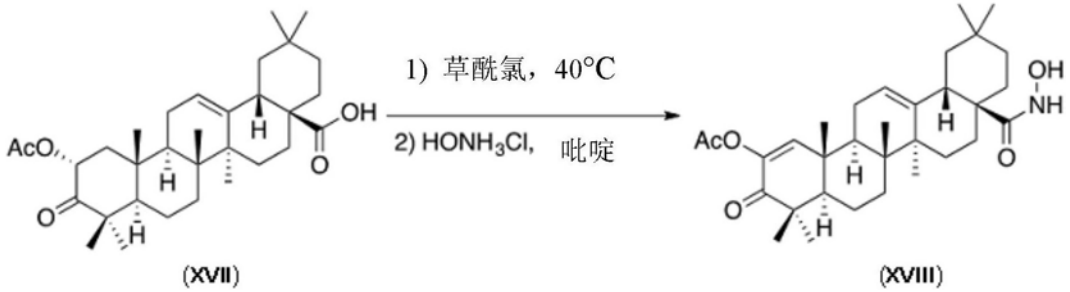
[0376] 在上文所述的一般合成方案(氧肟酸形成)之前,按下述进行已知的山楂酸衍生物(CAS编号1382923-75-2)C-2位置的羟基的乙酰化:

[0377] 

1382923-75-2 (XVII)

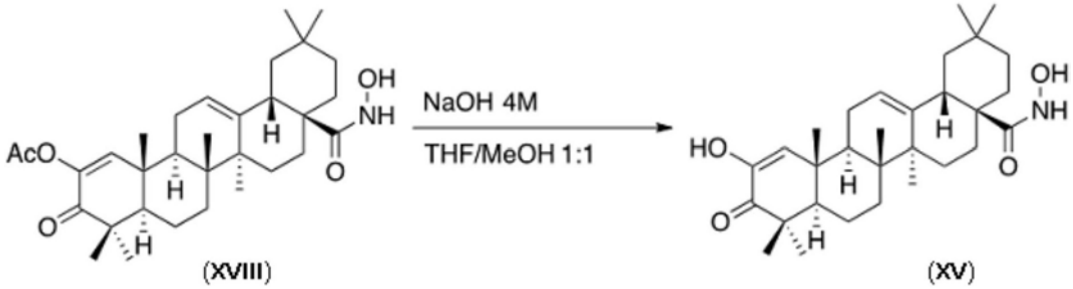
[0378] 向已知的山楂酸衍生物(1eq/mol)在无水吡啶(10mL/gr酸)中的溶液中,顺序加入乙酸酐(2eq/mol)和DMAP(0.1eq/mol)。将反应物在室温下搅拌直到起始物质消失(通过TLC控制),用甲醇猝灭,用2N H_2SO_4溶液稀释并用EtOAc萃取。将有机相用盐水洗涤,用Na_2SO_4干燥并在真空下挥发,以产生化合物XVII,无需进一步纯化。

[0379] 然后,按照上文所述的一般合成方案,使用化合物XVII按下述进行氧肟酸转化:

[0380] 

(XVII) (XVIII)

[0381] 然后,将化合物XVII按下述去乙酰化,以得到化合物XV:

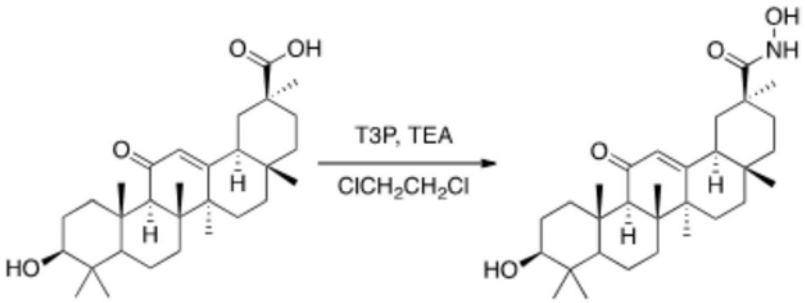
[0382] 

(XVIII) (XV)

[0383] 向化合物XVII(1eq/mol)在THF/MeOH 1:1中的溶液中,加入NaOH4N(50eq/mol)。将混合物在40℃加热过夜,用2N H_2SO_4溶液猝灭,并用EtOAc萃取。将有机相用盐水洗涤,用Na_2SO_4干燥并在真空下挥发。在硅胶上纯化粗制化合物。

[0384] 浅黄色固体(69%)。^1H NMR(300MHz,$CDCl_3$):d=6.31(s,1H),5.47(brt,1H),2.46(d,J=11.3Hz,1H),1.20(s,3H),1.19(s,3H),1.13(s,3H),1.10(s,3H),0.88(s,3H),0.86(s,6H),(仅报告了容易的峰);^{13}C NMR(75MHz,$CDCl_3$)d=201.0,176.4,145.2,143.8,127.8,123.2,53.7,46.1,45.5,44.0,43.1,42.4,40.9,40.1,38.4,33.9,32.9,32.0,30.7,27.1,27.0,25.8,23.6,23.4,21.9,20.8,19.6,18.7,17.1,14.2。

[0385] 通过使用T3P/三乙胺方案,在不需要使用保护基情况下从甘草次酸(GA)合成比较化合物XX(Ech-Chahad等人,Tetrahedron Letters 2005,46:5113-5115)。

[0386] 

GA (XX)

[0387] 在计算机芯片上(in silico)的测定

[0388] 实施例2.在计算机芯片上计算PHD2-结合亲和力。

[0389] 从PubChem(https://pubchem.ncbi.nlm.nih.gov/)获得或用MarvinSketch(ChemAxon,Cambridge,MA)设计分子结构。所用的受体模型是PDB参照4BQW。通过使用AutoDock4(Morris等人,J.Comp.Chem.2009Dec;30(16)2785-91)和Vina软件(Trott和Olson,J.Comp.Chem.2010Jan 30;31(2):455-61)以虚拟筛选工具PyRx(Wolf LK.Chem.&Eng.News 2009,87)计算结合特性。按照之前关于不同PHD2配体的一些结合位点的发现(Rabinowitz MH,J.Med.Chem.2013Dec 12;56(23):9369-4025),设置在受体分子表面周围对接的搜索空间。

[0390] 一旦已经进行分析,AutoDock Vina提供估算的结合亲和力值,其是分子间能(其是由于两个分子之间的相互作用)和扭转自由能罚分(其是由于这些分子采取的正确适配相互作用表面的构象而产生)的和。负值表示键是热动力学稳定的,而正值意指不稳定性。

[0391] 表I显示关于本发明所述的化合物以及阳性对照(IOX-2)和阴性对照(甘草次酸)的PHD2-结合能(Kcal/mol)。显示了使用结构4BQW(来自蛋白数据库(Protein Data Bank))

来自AutoDock-Vina的平均值。显示了来自三次独立的实验的平均值。

[0392]

化合物	B.E. AutoDock (Kcal/mol)
石竹素	-6.35
化合物 II	-6.33
化合物 III	-3.30
化合物 IV	-6.41
化合物 V	-3.35
化合物 VI	-5.27
桦木酸	-4.76
化合物 VII	-6.55
化合物 VIII	-5.77
化合物 IX	-4.33
化合物 X	-7.08
化合物 XI	-6.09
熊果酸	-5.74
化合物 XII	-6.01
化合物 XIII	-5.45
山楂酸	-3.20
化合物 XIV	-4.30
化合物 XV	-3.57
IOX 2	-9.05
甘草次酸	-1,24

[0393] 表I.三萜系化合物和衍生物针对PDH2的结合能。PHD2-结合能(Kcal/mol)。显示了使用结构4BQW(来自蛋白数据库(Protein Data Bank))的来自AutoDock/Vina的平均值。显示了来自三次独立的实验的平均值。

[0394] 搜索空间限制在残基H313、D315、H374、R383、Y303、Y310、Y329、I327、I256和M299周围的300 Å体积，通过沿着三个轴以10埃(Amstrong)为单位构建超过这些空间的格栅，提议所述残基为主要的结合位点(Rabinowitz MH,J.Med.Chem.2013Dec 12;56(23):9369-

4025)(图1)。发现关于化合物VII的获得的结合能为-6.55Kcal/mol,Ki 15.74uM(在298.15°K),分子间能为-7.38Kcal/mol,以及与原子位置的均方根偏差为0.0 Å,由此改善关于其天然前体桦木酸发现的结合特征,结合能为-4.76Kcal/mol。

[0395] 体外和体内测定

[0396] 实施例3.HIF-1α活性的选择性诱导

[0397] 为了研究不同化合物的生物学活性,在NIH-3T3-EPO-Luc细胞(表II)或HaCaT-EPO-luc细胞(图2)中进行HIF-1α反式激活测定。NIH3T3-EPO-luc和HaCaT-EPO-luc细胞已经稳定转染了质粒Epo-Luc质粒。EPO-低氧响应元件(Hypoxia Response Element,HRE)-荧光素酶受体质粒包含三个拷贝的融合在荧光素酶基因上的来自红细胞生成素基因启动子的HRE共有序列。将NIH3T3-EPO-luc细胞保持在37℃在包含5%CO_2的湿润气氛中的补充了10%胎牛血清(FBS)和1%(v/v)青霉素/链霉素的DMEM中。DFX购自Sigma-Aldrich(USA)。在测定前那天,接种细胞(在96孔平板中,1x10^4个/孔)。次日,将细胞用增加浓度的齐墩果酸(OA)、桦木酸(BA)、熊果酸(UA)、山楂酸(MA)、甘草次酸(GA)、式(I)、II至XV的化合物或比较化合物XX和CDDO-Me刺激。刺激六小时后,将细胞在室温(RT)在水平摇床上在25mM Tris-磷酸盐pH 7.8,8mM $MgCl_2$,1mM DTT,1%Triton X-100,和7%甘油中裂解15分钟。使用微量平板发光计(Berthold)按照荧光素酶测定试剂盒(Promega,Madison,WI,USA)的使用说明测量荧光素酶活性。计算RLU,并且使用下述等式,相对于150μM去铁胺(DFX)确定EC50和IRA(固有的相对活性)值:IRA系数=(EC_{50-DFX} x E_{max})/(EC_{50}x $E_{max-DFX}$),其中EC_{50}和E_{max}表示激动剂的EC_{50}和E_{max},并且EC_{50-DFX}和$E_{max-DFX}$表示标准激动剂DFX的EC_{50}和E_{max}值(表II)。

[0398] 用作本发明包括的化合物的合成模板的天然三萜系化合物(OA;齐墩果酸,BA;桦木酸;UA,熊果酸,和MA;山楂酸)都不能激活作为HIF-1α激活的替代标记的EPO启动子。相反,所有本发明的三萜系化合物衍生物清楚地激活HIF-1α途径(表II)。并且,如下表II所示,作为式I的齐墩果酸衍生物的化合物II至VI提供HIF途径的激活,而与此相反,在位置2(对应式I的位置B)包含氰化物基团的齐墩果衍生物,如CDDO-Me,不导致HIF途径的激活。

[0399] 由此,可以得出结论:在所述化合物中引入的化学修饰对于抑制PHD2的酶活性是至关重要的,并且因此对于激活HIF途径也是至关重要的,但对于化合物与蛋白的结合不是至关重要的。

[0400] 表II还显示了GA及其氧肟酸衍生物化合物XX不激活HIF-1α途径。因此,特别地,可以得出结论:在本发明定义的骨架位置28引入的化学修饰对于抑制PHD2的酶活性是至关重要的,并且因此对于激活HIF途径是至关重要的,但对于化合物与蛋白的结合不是至关重要的。

[0401]

化合物	功效 HIF-1α (IRA 系数)	效力 EC_{50} HIF-1α (μM)
OA		-(>50)
II	0,39	16.37
III	0,43	3.83
IV	0,15	11.39
V	0,16	6.71
VI	0,16	5.00
BA		-(>50)
VII	0,36	4.81
VIII	0,55	2.58
IX	0,34	2.41
X	0,48	3.24
XI	0,31	2.58
UA		-(>50)
XII	0,17	7.69
XIII	0,10	8.93
MA		-(>50)
XIV	0,10	7.10
XV	0,06	11.22
GA		-(>50)
XX		-(>50)
CDDO-Me		-(>50)

[0402] 表II.NIH-3T3-EPO Luc成纤维细胞中的HIF-1α反式激活测定。NIH3T3-EPO-luc细胞稳定转染了Epo-Luc质粒,所述质粒包含三个拷贝的融合在荧光素酶基因上的来自红细胞生成素基因启动子的低氧响应元件共有序列。显示了关于HIF-1α激活的功效和效力。

[0403] 接着,研究该化合物在另一种细胞类型如角质形成细胞系HaCaT-EPO-Luc中的活性。将细胞维持在37℃包含5%CO_2的湿润气氛中的补充了10%胎牛血清(FBS)和1%(v/v)青霉素/链霉素的DMEM中。在测定前一天接种细胞(在24孔平板中,$1x10^5$个/孔),然后用DFX(150μM)或增加浓度的化合物II至XV刺激6小时。然后,将细胞在25mMTris-磷酸盐pH 7.8,8mM $MgCl_2$,1mM DTT,1%Triton X-100和7%甘油中裂解。使用TriStar LB 941多方式微量平板读数仪(Berthold)按照荧光素酶测定试剂盒(Promega,Madison,WI,USA)的使用说明测定荧光素酶活性。上述测定由图2示例,其显示DFX和化合物II至XV在HaCaT-EPO-Luc细胞中的低氧模拟作用。将DFX(150μM)视为相对于未处理的细胞的100%诱导,提供作为激活的百分数的数据。与未处理的细胞相比,对所有三萜系化合物衍生物观察到荧光素酶活性的明显增加。

[0404] 为了进一步研究本发明所述的化合物的靶向选择性,分析了天然三萜系化合物(OA;BA,UA和MA)和本发明公开的式(I)的化合物II至XV、比较化合物CDDO-Me对NF-κB抑制、STAT-3抑制、Nrf2激活和TGR5激活的影响。对于该研究,分别使用细胞系NIH-3T3-KBF-Luc、HeLa-STAT3-Luc、HaCaT-ARE-Luc和CHO-TGR5-CRE-luc。NIH3T3-KBF-Luc细胞系稳定转染质粒KBF-Luc质粒,所述质粒包含三个拷贝的融合在驱动荧光素酶基因的最小猿猴病毒40启动子上的NF-κB结合位点(来自主要组织相容性复合物启动子)。将细胞($1x10^4$个/孔)接种

在96孔平板中，用增加浓度的化合物II至XV处理15分钟，然后用30ng/ml TNFα刺激。6小时后，如上文所示测量细胞裂解物中的荧光素酶活性。计算RLU，并且将结果表示为由TNFα诱导的NF-κB活性(100％激活)的抑制百分数(表III)。

[0405]

化合物	IC_{50} NF-κB (μM)	IC_{50} STAT3 (μM)	EC_{50} NRF2 (μM)	IC_{50} NRF2 (μM)	EC_{50} TGR5 (μM)
OA	-(>50)	-(>50)	-(>50)	-(>50)	18.90
II	-(>50)	-(>50)	-(>50)	-(>50)	-(>50)
III	-(>50)	-(>50)	23.93	-(>50)	-(>50)
IV	-(>50)	-(>50)	10.04	-(>50)	-(>50)
V	-(>50)	-(>50)	-(>50)	-(>50)	-(>50)
VI	-(>50)	-(>50)	-(>50)	-(>50)	-(>50)
BA	-(>50)	-(>50)	9.02	-(>50)	22.15
VII	-(>50)	-(>50)	-(>50)	-(>50)	-(>50)
VIII	-(>50)	-(>50)	-(>50)	-(>50)	9.62
XI	-(>50)	-(>50)	-(>50)	-(>50)	17.50
X	-(>50)	-(>50)	-(>50)	-(>50)	-(>50)
XI	-(>50)	-(>50)	-(>50)	-(>50)	17.50
UA	-(>50)	-(>50)	38.36	-(>50)	11.46
XII	-(>50)	-(>50)	-(>50)	-(>50)	5.91
XIII	-(>50)	-(>50)	-(>50)	-(>50)	-(>50)
MA	-(>50)	-(>50)	-(>50)	-(>50)	10.19
XIV	-(>50)	-(>50)	-(>50)	-(>50)	-(>50)
XV	(>50)	(>50)	(>50)	(>50)	(>50)
CDDO-Me	1.20	2,38	40,94	0.06	(>50)

[0406] 表III.对NF-κB、STAT-3、Nrf2和TGR5途径的影响。

[0407] 化合物II至XV，OA；BA，UA，MA和比较化合物CDDO-Me对NF-κB抑制(IC50)、STAT-3抑制(IC50)、Nrf2激活(EC50)与抑制(IC50)以及TGR5激活(EC50)的影响。我们分别使用下述细胞系：NIH-3T3-KBF-Luc，HeLa-STAT3-Luc，HaCaT-ARE-Luc和CHO-TGR5-CRE-luc。显示了IC50和EC50数据。

[0408] HeLa-STAT3-luc细胞稳定转染了质粒4xM67pTATA TK-Luc。将细胞($20x10^3$个细胞/ml)接种在96孔平板中，采用增加浓度的化合物II至XIV和比较化合物CDDO-Me处理15分钟，然后用IFN-γ 25IU/ml刺激。6小时后，如上文所示测量细胞裂解物中的荧光素酶活性。计算RLU，并且结果表示为由IFN-γ诱导的STAT3活性(100％激活)的抑制百分数(表III)。HaCaT-ARE-Luc细胞系包含Nqo1抗氧化剂响应元件(ARE)-Luc报道子质粒。ARE被CNC家族因子的所有成员(Nrf1，Nrf2，Nrf3和p45NF-E2)激活。将细胞以$25x10^3$个细胞/孔的浓度在96孔平板中在CO_2培养箱中在37℃培育。为了诱导Nrf2激活，将细胞用增加浓度的化合物II至XV和比较化合物CDDO-Me处理6小时。作为阳性对照，将细胞用0.02mM抗氧化剂叔丁基氢醌(TBHQ)处理。如上文所示测定细胞裂解物中的荧光素酶活性，并且计算EC50(表III)。CHO-TGR5-CRE-Luc细胞稳定转染pTGR5和CRE-Luc。CRE-反应性荧光素酶构建体在最小(m)CMV启动子和CRE转录响应元件(TRE)串联重复的控制下编码萤火虫荧光素酶报道子基因，并且用于监测由TGR5激动剂激活的cAMP信号传导途径。将细胞($1x10^4$个/孔)接种在96孔平板中，用增加浓度的化合物II至XV和CDDO-Me处理6小时。作为阳性对照，将细胞用10μM LCA(石胆

酸)处理。如上文所示测量细胞裂解物中的荧光素酶活性,并且计算EC50(表III)。

[0409] 所述化合物都不抑制分别由TNFα和IFNγ诱导的NF-κB和STAT3途径。另外,所述化合物都不激活Nrf2途径,仅化合物VIII、XI和XII表现出TGR5激动活性(表III)。相反,CDDO-Me清楚地抑制NF-κB和STAT-3信号传导途径并且激活Nrf2途径。该结果还证明,氰化物基团对于某些生物学活性是至关重要的,但不是激活HIF-1α途径所需要的(表III)。

[0410] 实施例4.三萜系化合物衍生物稳定HIF-1α和HIF-2α

[0411] 为了获得本发明所述的化合物对调节HIF-1α稳定化的深刻了解,研究了对不同细胞类型中HIF-1α表达的影响。将人少突胶质细胞MO13.3细胞用150μDFX或10μM齐墩果酸(OA),化合物II,III,IV,V,VI(图3A),桦木酸(BA),化合物VII,VIII,IX,X,XI(图3B),熊果酸(UA),山楂酸(MA),化合物XII,XIII,XIV和XV(图3C)刺激3小时。之后,将细胞用PBS洗涤并用50μl补充了10mM NaF、1mM Na_3VO_4、10μg/ml亮肽素(leupeptine)、1μg/ml胃蛋白酶抑制剂和抑肽酶与1μl/ml饱和的PMSF的NP-40缓冲液(50mM Tris-HCl pH 7.5,150mM NaCl,10%甘油和1%NP-40)温育。离心后,将上清与SDS样品缓冲液混合,并在95℃煮沸。将蛋白在8-10%十二烷基硫酸钠聚丙烯酰胺凝胶(SDS-PAGE)中电泳并转移到聚偏氟乙烯膜上(每张膜,20V和30min)。用在TBST缓冲液中的脱脂乳或BSA封闭后,加入一级抗体。将洗涤的膜用适当的与辣根过氧化物酶偶联的二级抗体温育,所述二级抗体由增强的化学发光系统(USB)检测。针对HIF-1α的抗体(610959)来自BD Biosciences,并且抗体抗-β-肌动蛋白(AC-74)购自Sigma-Aldrich(Saint Louis,MO,USA)。

[0412] 在正常含氧量条件下(21%O_2),本发明所述的所有化合物都升高HIF-1α蛋白水平。诱导的程度与去铁胺(desferrioxamine)(DFX)的诱导程度相当,去铁胺为一种已知稳定HIF-1α的铁螯合剂(图3A,3B和3C)。

[0413] 接着,将人胚肾293细胞(293T)用增加浓度的化合物VII或用DFX(150μM)刺激3小时。之后,如图3所示,进行蛋白分离和蛋白质印迹。针对HIF-1α的抗体(610959)来自BD Biosciences(USA),抗体抗-PHD1(ab80361)和抗-PHD2(ab109088)来自Abcam(Cambrigde,UK),抗体抗-β-肌动蛋白(AC-74)购自Sigma-Aldrich(Saint Louis,MO,USA)。

[0414] 结果清楚地显示,作为本发明所述的化合物的代表的化合物VII稳定HIF-1α表达,而不影响PDH1和PDH2的表达(图4)。

[0415] 由于PDH2和PDH3还调节HIF-2α的表达,因此研究了化合物VII对获自Innoprot SL(Spain)(参照p10472)的人胰岛来源的前体细胞(hIPC)中的HIF-2α稳定化的影响。将hIPC用增加浓度的化合物VII或用DFX(150μM)刺激3小时。之后,如图3所示,进行蛋白分离和蛋白质印迹。针对HIF-2α(ab8365)和PHD3(ab30782)的抗体来自Abcam(Cambrigde,UK),抗体抗-β-肌动蛋白(AC-74)购自Sigma-Aldrich(Saint Louis,MO,USA)。(图5)。

[0416] 结果清楚地显示,作为本发明所述的化合物的代表的化合物VII稳定HIF-2α表达,而不影响PHD3的表达(图5)。

[0417] 总之,结果表明化合物VII结合PDH2,抑制其活性,并且因此稳定HIF-1α和HIF-2α蛋白水平。

[0418] 实施例5.化合物VII诱导血管生成。

[0419] 为了检测化合物VII刺激在生理学模型中的功能结果,测量作为血管生成模型的内皮细胞管形成。提供CellPlayer™ GFP AngioKit-96(Essen BioScience Inc.,Welwyn

Garden City,UK)作为在管形成最早阶段的人基质(正常人皮肤成纤维细胞,NHDF)和内皮细胞(HUVEC)的生长共培养物。按照供应商方案进行CellPlayer 96孔动力学血管生成测定。简言之,将慢病毒感染的绿色荧光蛋白(GFP)-HUVEC与正常的人皮肤成纤维细胞在96孔微量平板中共同培养。将平板放置在IncuCyte中,并且,每6小时自动获得相和荧光照片,持续7天。在第1天,将化合物VII(1和2,5μM)或VEGF(10ng/ml)添加到内皮管网络上,并且在整个实验过程中保持。使用Essen BioScience血管生成分析组件(Essen BioScience Angiogenesis Analysis Module)将7天测定期间的管形成定量。该组件提供多个测定度量,包括管长度和分支点形成,其用来评估对网络形成的血管生成作用。简言之,分析荧光照片以产生紧密类似于体外网络的分段罩物。然后提炼该罩物以特别鉴定管形成事件,并且使用IncuCyte和GraphPad Prism软件(La Jolla,CA)将动力学反应绘图。

[0420] 在图6中,显示了化合物VII 1μM以及阳性对照(rFGF;10ng/ml和VEGFA;10ng/ml)显著增加HUVEC细胞中的网络长度。

[0421] 实施例6.化合物VII提高红细胞生成素(EPO)的血浆水平。

[0422] 红细胞生成素(EPO)是最早记载的且最有效的HIF靶基因之一;在转录水平上被正调节。此处,检验了化合物VII在体内调节EPO水平的能力。将十六周龄的C57BL/6雄性小鼠用桦木酸(60mg/kg)或化合物VII(30mg/kg或60mg/kg)腹膜内(i.p.)处理。在处理后4小时,在全身麻醉下采集血液样品,并且使用小鼠EPO ELISA试剂盒(R&D Systems)按照供应商的使用说明定量血浆中的EPO循环水平。EPO值表示平均值±SEM(n=3)。

[0423] 如图7所示,在小鼠中体内施用作为本发明所述的化合物的代表的化合物VII(30和60mg/kg/天)强烈增加了循环的EPO血浆水平。相反,化合物VII的母体桦木酸(BA)不影响血浆中的EPO水平。

[0424] 实施例7.化合物VII对纹状体细胞中3-NP-诱导的细胞毒性的影响和小鼠中3-NP-诱导的亨廷顿病的预防作用。

[0425] 7.1:化合物VII对纹状体细胞中3-NP-诱导的细胞毒性的影响

[0426] 为了研究纹状体Q7和Q111细胞中的HIF激活是否足以保护纹状体神经元(提供神经保护作用),检验了化合物VII对3-NP诱导的死亡的影响。具体而言,研究用化合物VII预处理6小时对暴露于3-硝基丙酸(3-NP)24小时的$STHdh^{Q7/Q7}$(纹状体Q7)和$STHdh^{Q111/Q111}$(纹状体Q111)细胞的影响。

[0427] $STHdh^{Q111/Q111}$细胞表达突变形式的亨廷顿蛋白,并且$STHdh^{Q7/Q7}$细胞表达这种蛋白的野生型形式。将由$Hdh^{Q111/Q111}$(突变体)和$Hdh^{Q7/Q7}$(野生型)敲入小鼠同窝幼崽的E14纹状体原基建立的克隆纹状体细胞系用转导tsA58/U19大T-抗原的复制缺陷型反转录病毒永生化(Trettel F.等人,Hum Mol Genet.2000;9:2799-2809)。将纹状体Q7和Q111细胞保持在含有25mM D-葡萄糖、1mM L-谷氨酰胺、10%胎牛血清(FBS)、1mM丙酮酸钠和400μg/mL遗传霉素的Dulbecco's改良Eagle培养基(DMEM)中,并且在33℃用5%CO_2温育。

[0428] 3-硝基丙酸(3-NP)是线粒体复合物II酶的有效的不可逆抑制剂,并且导致线粒体功能障碍和氧化性应激。

[0429] 将$STHdh^{Q7/Q7}$和$STHdh^{Q111/Q111}$细胞(在96孔平板中,10^4个细胞/孔)用YOYO-1(Life Technologies)温育,然后用3-NP(10mM)和或DFX(50μM)(作为PHD抑制的阳性对照)处理。将YOYO-1稀释在细胞培养基中,并且添加到实验孔和对照孔中至终浓度为0.1μM。YOYO-1是细

胞不渗透氰化物二聚体核酸染料,其仅可进入具有损坏的质膜的细胞并且将核DNA荧光染色。损坏的细胞对YOYO-1的摄入与YOYO-1荧光的增加相关联。将处理的细胞放置在Incucyte FLR成像系统中,并且在3-NP处理24小时后测量YOYO-1荧光。在温育期间之后,使用Incucyte FLR软件进行对象计数分析,以计算YOYO-1荧光阳性细胞的总数和全部包含DNA的对象(终点)。对每个处理组,通过将YOYO-1荧光阳性对象的数目除包含DNA的对象总数来计算细胞毒性指数,并且转化为细胞死亡的百分数。

[0430] $STHdh^{Q111/Q111}$细胞比$STHdh^{Q7/Q7}$细胞对暴露于3-NP更敏感,并且化合物VII清楚地提供针对3-NP-诱导的细胞毒性的显著水平的神经保护作用(图8)。尽管程度较小,但是DFX也保护细胞免受3-NP的细胞毒性活性。

[0431] 7.2:在小鼠中预防3-NP诱导的亨廷顿病

[0432] 用3-硝基丙酸(3-NP)使小鼠中毒导致无数的神经学、生物化学和组织学作用,其暗示亨廷顿病(HD)病理学的一些方面。与对照动物(没有3-NP中毒的)相比,3NP-处理的小鼠在后肢抱握、张力失调、脊柱后凸和一般的运动活动方面表现出高评分。

[0433] 通过六次腹膜内(i.p.)注射3-硝基丙酸(3NP)(30mg/kg;每12小时注射一次,在PBS中制备)在16周龄的C57BL/6雄性小鼠(Harlan Ibérica,巴塞罗那,西班牙)中诱导纹状体神经变性。将3NP-处理的动物和没有损伤的对照组(注射PBS)用于使用桦木酸(30mg/Kg)和化合物VII(30mg/Kg)的药理学研究。处理由5次腹膜内(i.p.)注射组成,每24小时注射50mg/kg测试化合物或媒介物(盐水缓冲液中10%DMSO加6,2%Tween 20),第一次注射在第一次3NP注射前24小时,其余的剂量给药在3NP注射前30分钟。对小鼠进行行为测试,以确定它们的神经学状态。评价一般的运动活动、后肢抱握和张力失调以及躯干张力失调。所有的行为测试在药物注射之前进行,以避免研究的化合物的急性效应。所有的行为测试在药物注射之前进行,以避免研究的化合物的急性效应,并且所有的动物在最后一次3NP注射后12小时安乐死。一旦安乐死,将动物解剖,并且快速摘下它们的脑。右半球用来解剖纹状体,将其在RNAlater(Sigma-Aldrich,德国)中快速冷冻,以通过实时PCR分析炎性标记。将左半球在新鲜的4%低聚甲醛(在0.1M磷酸盐缓冲的盐水中)中在4℃固定48小时,并且包埋在石蜡中用于组织学分析。

[0434] 图9显示化合物VII清楚地减轻由3-NP中毒诱导的临床症状。桦木酸(BA)也表现出一些神经保护活性,但是程度较低。

[0435] 接着,还使用3NP-损伤的小鼠的纹状体实质进行一些与炎症和神经变性相关的组织学和分子标记分析,所述标记在该实验模型中受到影响。这些3NP-处理的动物的纹状体实质表现出Nissl-染色细胞的重要减少,这表明由3NP引起的神经元死亡的重要程度,通过用化合物VII处理明显地防止了该神经元死亡,但是使用桦木酸(BA)没能防止(图10)。

[0436] 另外,化合物VII-介导的神经保护作用与减少的3NP-诱导的小胶质细胞增生和星形胶质细胞增生相关,其由Iba1和GFAP免疫组织化学确定(图11)。对于该测试,将用3-硝基丙酸(3-NP)中毒的小鼠用于确定小胶质细胞激活和星形胶质细胞增生,包括使用未中毒小鼠的对照(3NP,3NP+BA和3NP+化合物VII)。通过对不同组小鼠的脑切片的免疫染色来确定Iba-1和神经胶质原纤维酸性蛋白(GFAP)表达。使用Image J软件进行不同标记的定量。图11显示了小胶质细胞($Iba1^{+}$)和星形胶质细胞($GFAP^{+}$)的总平均数。

[0437] 最后,在3NP-损伤的小鼠中,炎性酶COX-2和iNOs的表达显著上调,伴有促炎性细

胞因子IL-1β和IL-6的表达增加。与3NP+媒介物小鼠相比，在3NP+化合物VII(30mg/kg)处理的小鼠中，关于COX-2(图12D)、IL-1β(图12B)、IL-6(图12A)和iNOS(图12C)的mRNA表达被下调。桦木酸(BA)处理(30mg/kg)也抑制炎性标记的表达(如图12所示)。使用$2^{-\Delta\Delta Ct}$方法计算表达水平。值表示为每组6只动物的平均值±SEM。

[0438] 实施例8.用于本发明的实时定量PCR(实施例7)。

[0439] 使用RNeasy脂质组织迷你试剂盒(RNeasy Lipid Tissue Mini Kit,Qiagen,GmbH)从纹状体(3NP模型)分离总RNA。通过在260nm处的光谱测定法来定量提取的RNA的总量和通过其在260与280nm的吸光度值之间的比例来定量纯度。去除基因组DNA以消除DNA污染。使用iScript™ cDNA合成试剂盒(Bio-Rad,Hercules,CA,USA)从多至1μg的总RNA(来自每组至少3只动物的汇集)合成单链互补DNA。将反应混合物在-20℃保持冷冻，直到用于酶促扩增时。使用iQ™ SYBR Green Supermix(Bio-Rad)来定量COX-2、IL-6、IL-1β和iNOS的mRNA水平。使用CFX96实时PCR检测系统(Bio-Rad)进行实时PCR。使用GAPDH看家基因来使每份样品中的mRNA表达水平标准化。使用$2^{-\Delta\Delta Ct}$法计算表达水平。表IV提供了寡核苷酸引物的序列。

[0440] 表IV.用于定量聚合酶链式反应的小鼠引物序列的列表。

[0441]

基因	正向	反向
IL-6	5′-GAACAACGATGATGCACTTGC-3′	5′-TCCAGGTAGCTATGGTACTCC-3
iNOS	5′-AACGGAGAACGTTGGATTTG-3′	5′-CAGCACAAGGGGTTTTCTTC-3′
COX-2	5′-TGAGCAACTATTCCAAACCAGC-3′	5′-GCACGTAGTCTTCGATCACTATC-3′
IL-1β	5′-CTCCACCTCAATGGACAGAA-3′	5′-GCCGTCTTTCATTACACAGG-3′
GAPDH	5′-TGGCAAAGTGGAGATTGTTGCC-3′	5′-AAGATGGTGATGGGCTTCCCG-3′

[0442] 所述结果证实了本发明所述的化合物用于临床管理其治疗响应于HIF激活的病症和疾病的应用，所述病症和疾病如卒中，脑瘫，创伤性损伤，神经变性疾病(如多发性硬化、亨廷顿病、阿尔茨海默病和帕金森病)，IBD，心肌缺血-再灌损伤，急性肺损伤，器官移植，急性肾损伤和动脉病。

[0443] 实施例9.化合物VII对糖尿病和代谢病理学的高脂肪饮食(HFD)模型的作用。

[0444] 用高脂肪饮食喂养的小鼠是用于受损的葡萄糖耐量(IGT)和2型糖尿病的常用模型。实验中使用八周龄的雄性C57BL/6小鼠(Charles River-France)。将小鼠保持在控制条件下[12h光/暗周期;温度20℃(±2℃)和40-50％相对湿度]，自由获得自来水和随意获得标准啮齿动物食物。适应1周后，将小鼠分成两组，并随意用标准饮食-CD-(Code U8220G10R,SAFE Diets,Augy,法国)或高脂肪饮食-HFD-(D12451;Research Diets,New Brunswick,NJ)喂养，以诱导肥胖15周(饮食-诱导的肥胖，DIO)。为了评估化合物VII在该DIO模型中潜在有益的代谢作用，通过从饮食暴露的第12周至第15周每24小时进行腹膜内(i.p.)注射而进行所述化合物(30mg/kg体重)的药理学施用。对照动物接受相应的媒介物注射(1:1:18乙醇:克列莫佛(Cremophor):盐水)。

[0445] 为了确保HFD喂养方案的有效性并评估化合物在减轻代谢表型方面的功效，使用EchoMRI™700分析仪(Houston,TX,软件v.2.0)通过QMR(定量磁共振)进行脂肪和瘦肉量的身体组成分析。在这种情形中，在实验期间的三个时间点进行MRI扫描:在开始饮食暴露之

前，在喂养方案12周后(同时开始治疗)和在实验程序结束时(在饮食暴露的第15周)。另外，在处死前五天，对过夜禁食的小鼠进行葡萄糖耐量测试(GTT)。为此目的，以2mg葡萄糖/g体重对小鼠腹膜内注射，并且在注射前和注射后30、60和120min，使用Accu-Chek Advantage®(Roche)血糖仪测量血糖。在实验结束时，将小鼠处死，并且使用EDTA-包被的管采集血液样品，并将血浆保存在-80℃。使用酶比色试剂盒(QCA，巴塞罗那，西班牙)测量血浆甘油三酯。

[0446] 我们发现化合物VII减少：体重增加(图13A)、脂肪量增加(图13B)和肥胖(图13C)。并且，化合物VII改善HFD小鼠的糖耐量(图14)并使血浆甘油三酯水平正常化(图15)。我们的结果与之前的报道一致，之前的报道表明PDH抑制剂改善由HFD诱导的小鼠肥胖和糖尿病模型中的葡萄糖和脂质代谢(Rahtu-Korpela等人，Diabetes.2014Oct;63(10):3324-33)。因此，所述结果表明：本发明的化合物可用于治疗糖尿病或其他相关的代谢病理学，如高甘油三酯血症。

序列表

<110> 维瓦赛尔生物技术西班牙有限公司

<120> 三萜系化合物衍生物

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<150> EP16193684.4
<151> 2016-10-13

<160> 10

<170> BiSSAP 1.3.6

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<212> DNA
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<220>
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[0001]

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<210> 4
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[0002]

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[0003]

<220>
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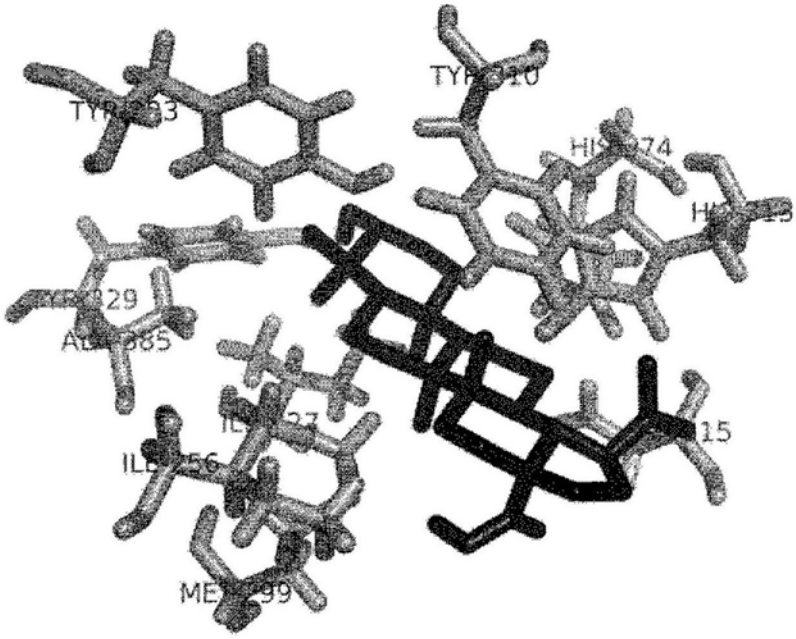
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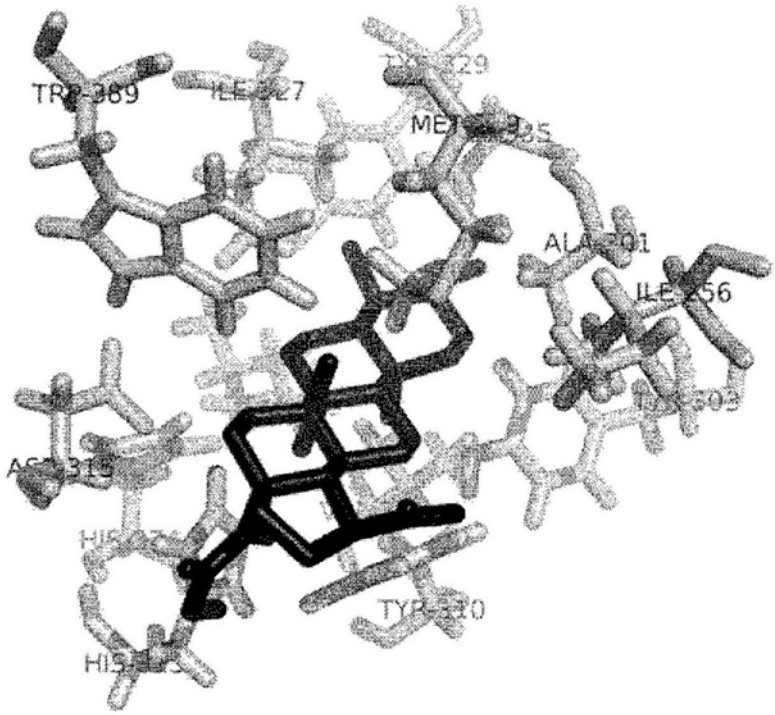
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<400> 10
aagatggtga tgggcttccc g 21


TYR-210
HIS-374
ALA-385
ILE-256
MET-299

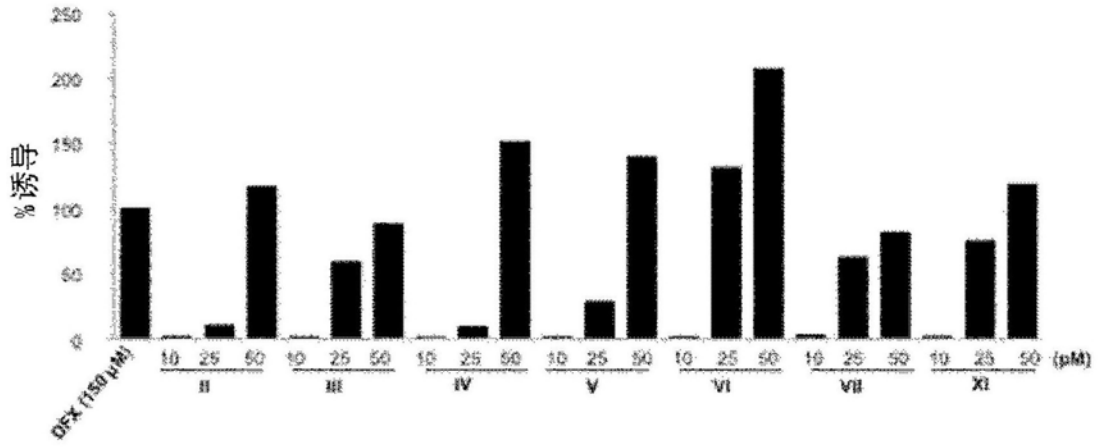
1A


TRP-389
ILE-427
MET-435
ILE-256
TYR-210

1B

图1

2A


% 诱导
250
200
150
100
50
0
DFX (150 μM)
10 25 50 (μM)
II
III
IV
V
VI
VII
XI

2B

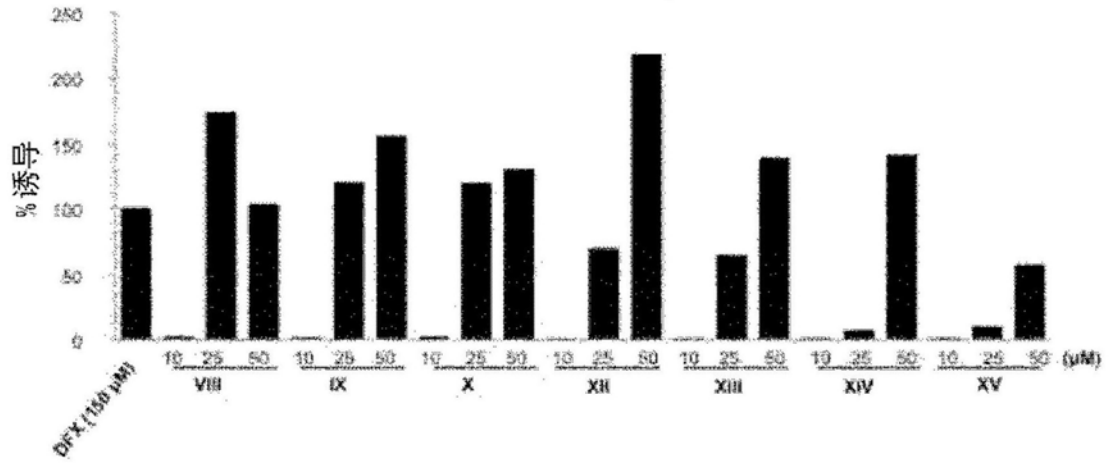

% 诱导
250
200
150
100
50
0
DFX (150 μM)
10 25 50 (μM)
VIII
IX
X
XII
XIII
XIV
XV

图2

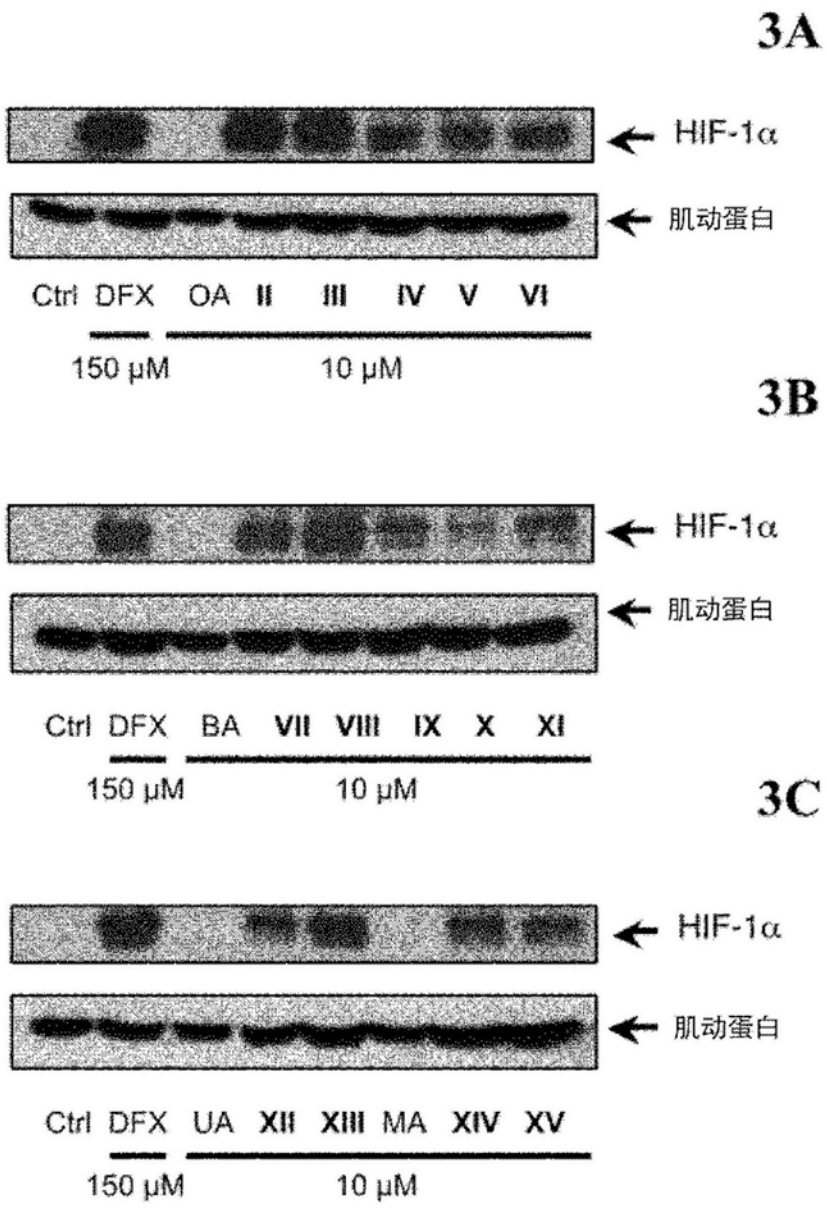

3A
HIF-1α
肌动蛋白
Ctrl DFX OA II III IV V VI
150 μM
10 μM
3B
HIF-1α
肌动蛋白
Ctrl DFX BA VII VIII IX X XI
150 μM
10 μM
3C
HIF-1α
肌动蛋白
Ctrl DFX UA XII XIII MA XIV XV
150 μM
10 μM

图3

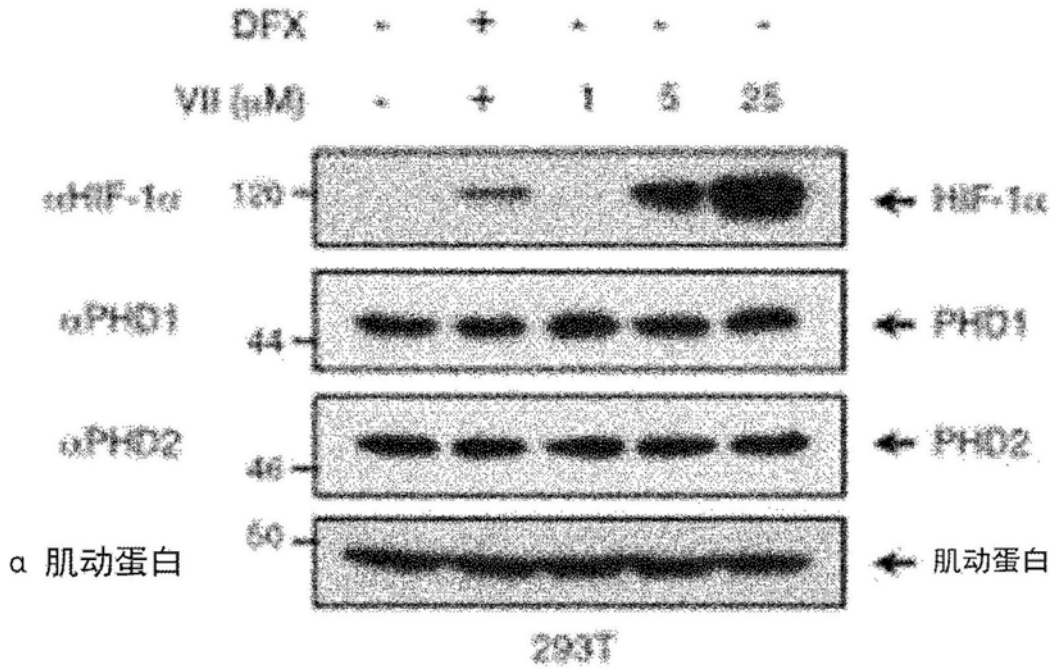

DFX - + - - -
VII (μM) - + 1 5 25
αHIF-1α
120
HIF-1α
αPHD1
44
PHD1
αPHD2
46
PHD2
α 肌动蛋白
50
肌动蛋白
293T

图4

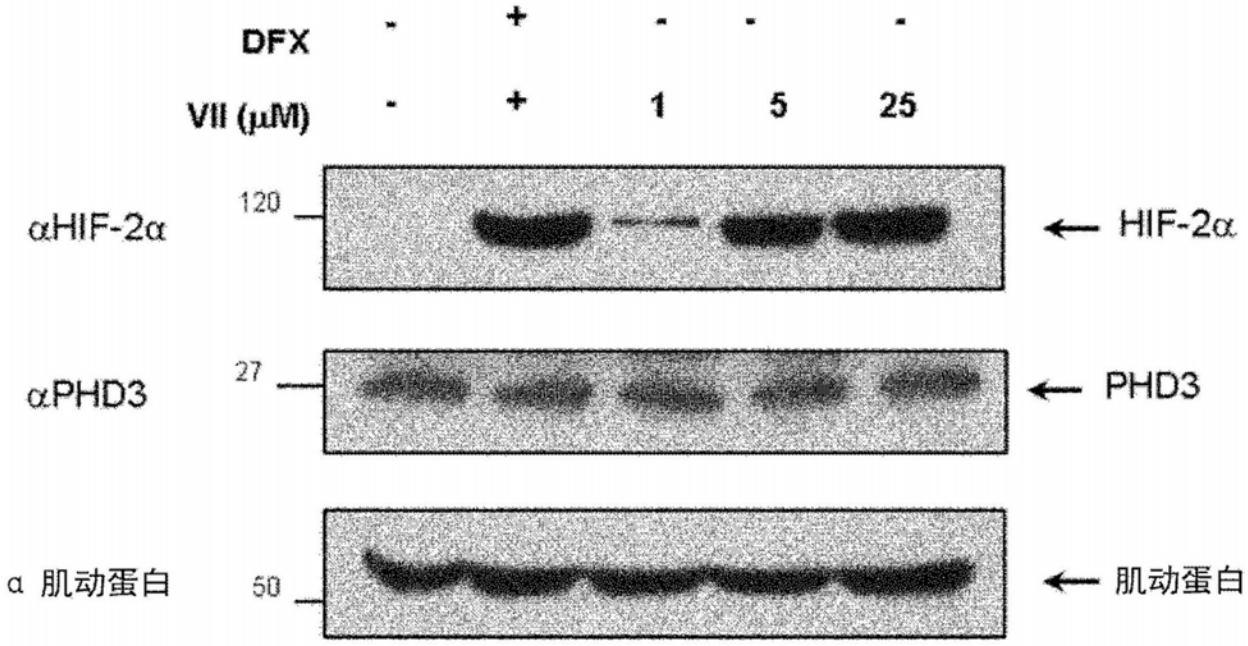

DFX - + - - -
VII (μM) - + 1 5 25
αHIF-2α
120
HIF-2α
αPHD3
27
PHD3
α 肌动蛋白
50
肌动蛋白

图5

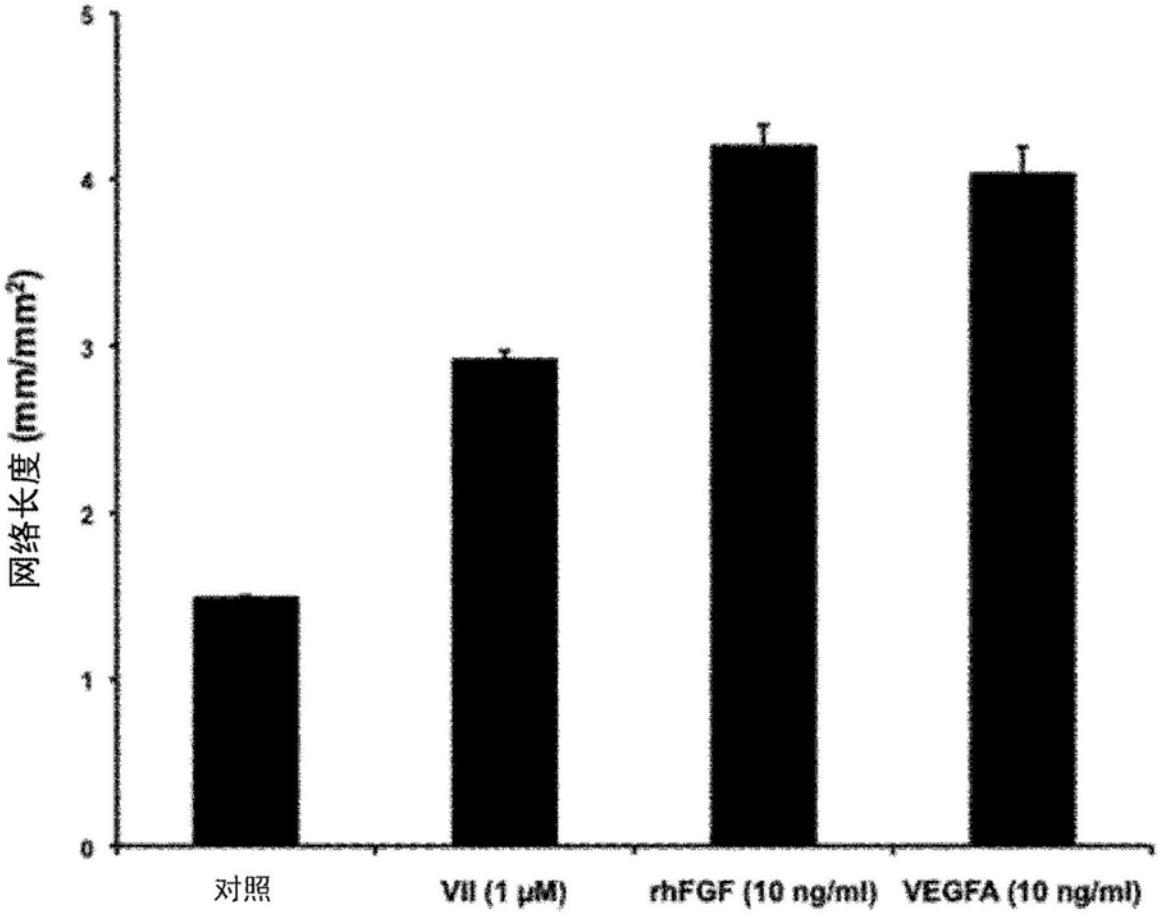


图6

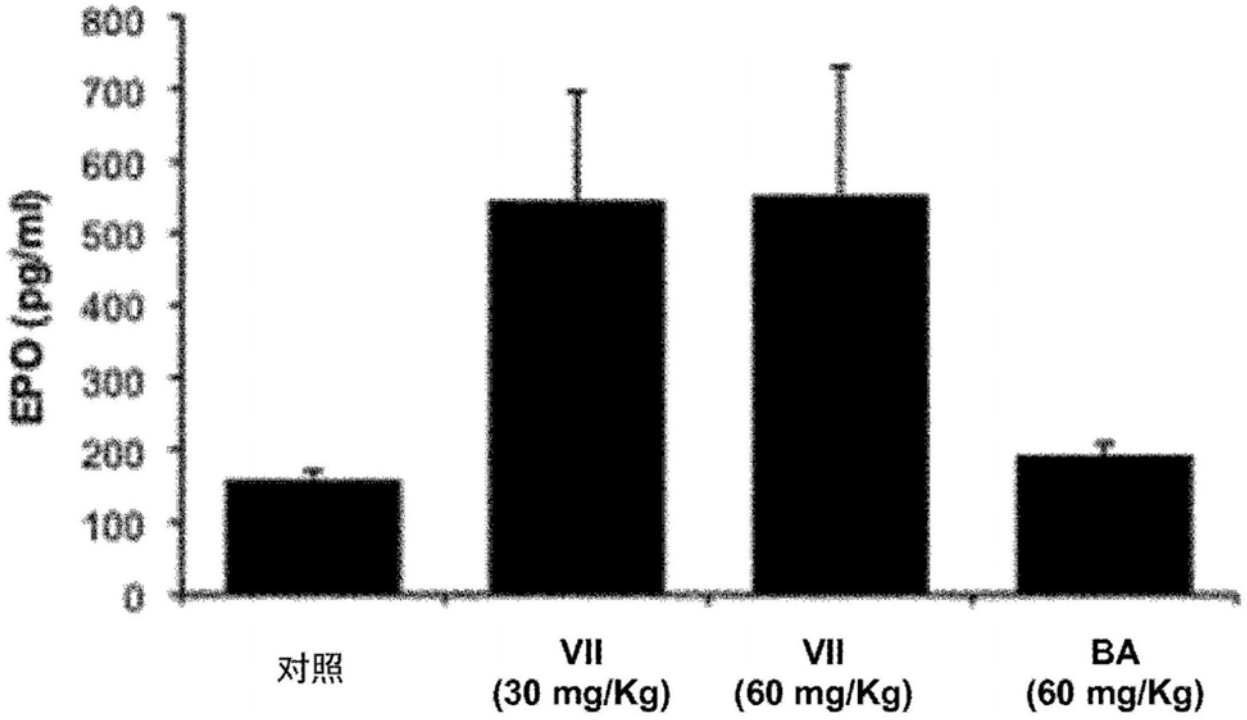

EPO (pg/ml)
800
700
600
500
400
300
200
100
0
对照
VII (30 mg/Kg)
VII (60 mg/Kg)
BA (60 mg/Kg)

图7

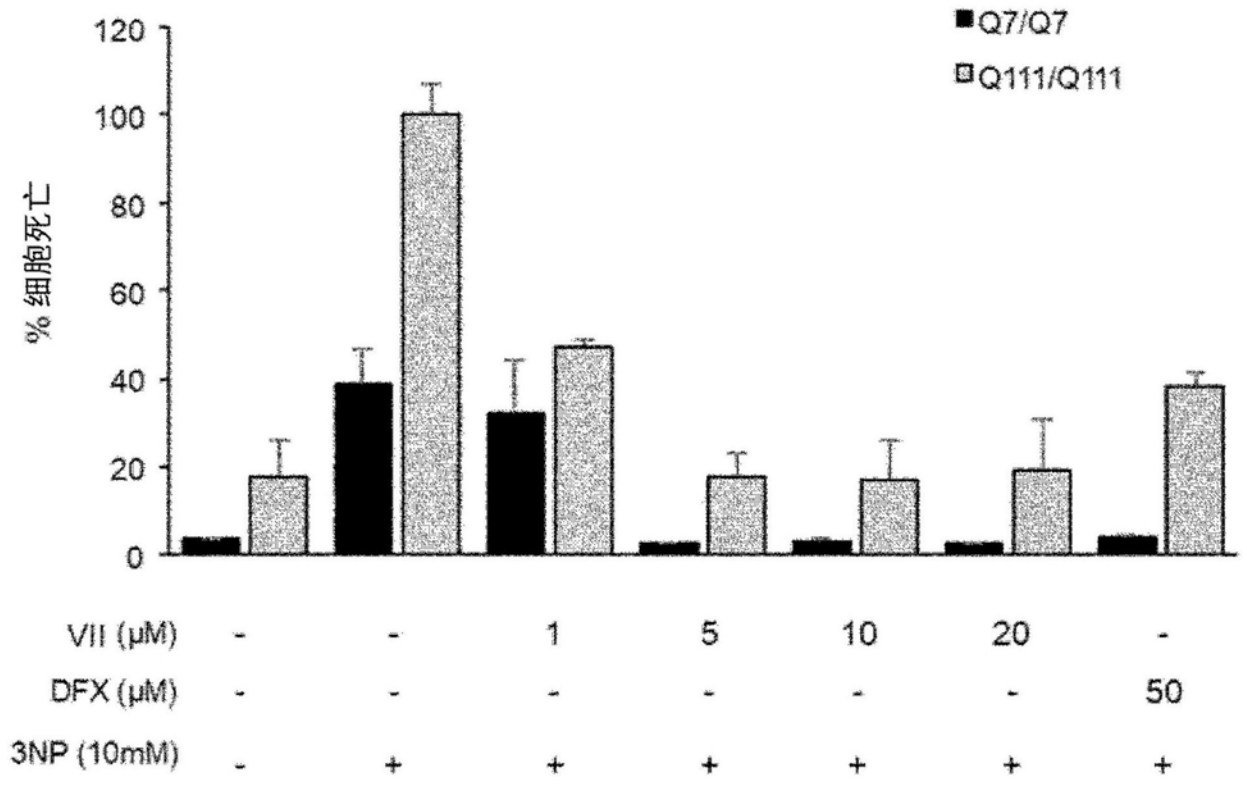

Q7/Q7
Q111/Q111
% 细胞死亡
120
100
80
60
40
20
0
VII (μM) - - 1 5 10 20 -
DFX (μM) - - - - - - 50
3NP (10mM) - + + + + + +

图8

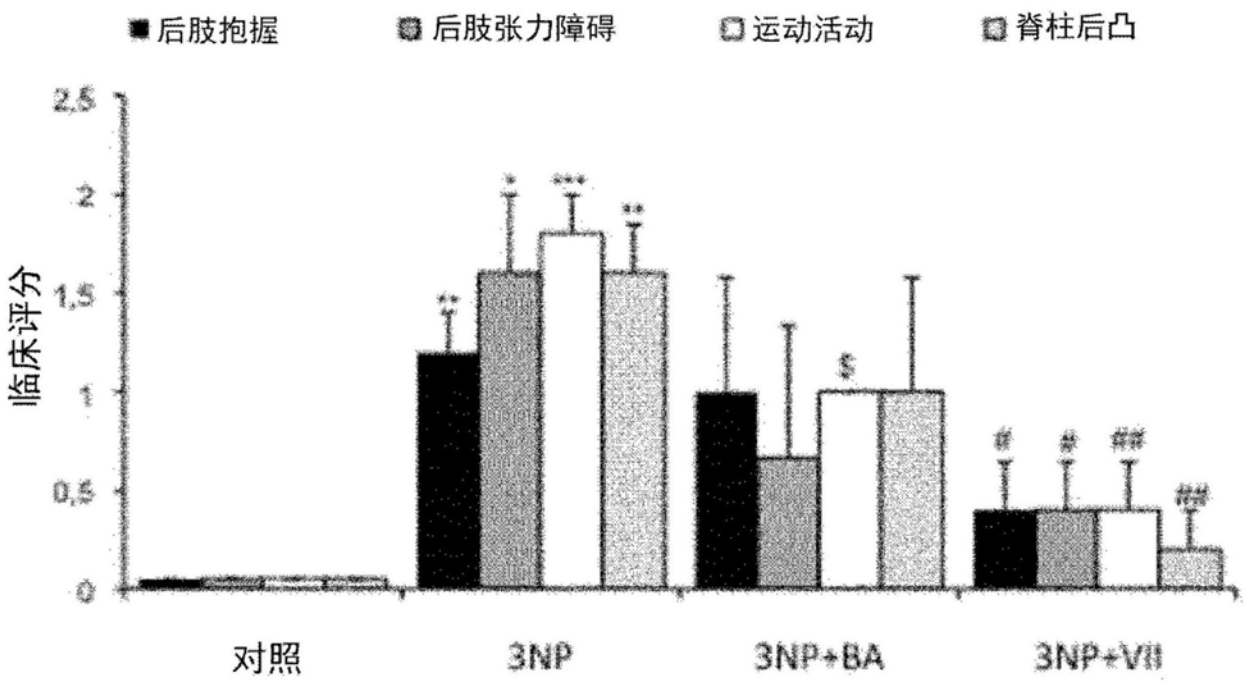

后肢抱握
后肢张力障碍
运动活动
脊柱后凸
临床评分
2.5
2
1.5
1
0.5
0
对照
3NP
3NP+BA
3NP+VII

图9

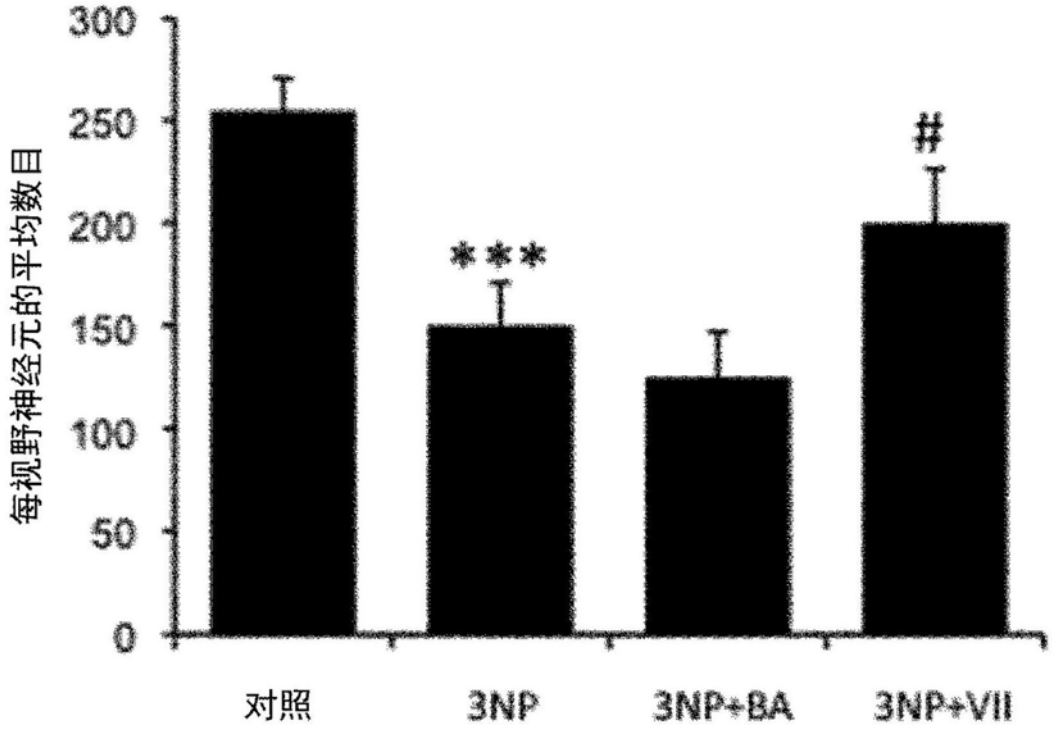

每视野神经元的平均数目
300
250
200
150
100
50
0
对照
3NP
3NP+BA
3NP+VII

图10

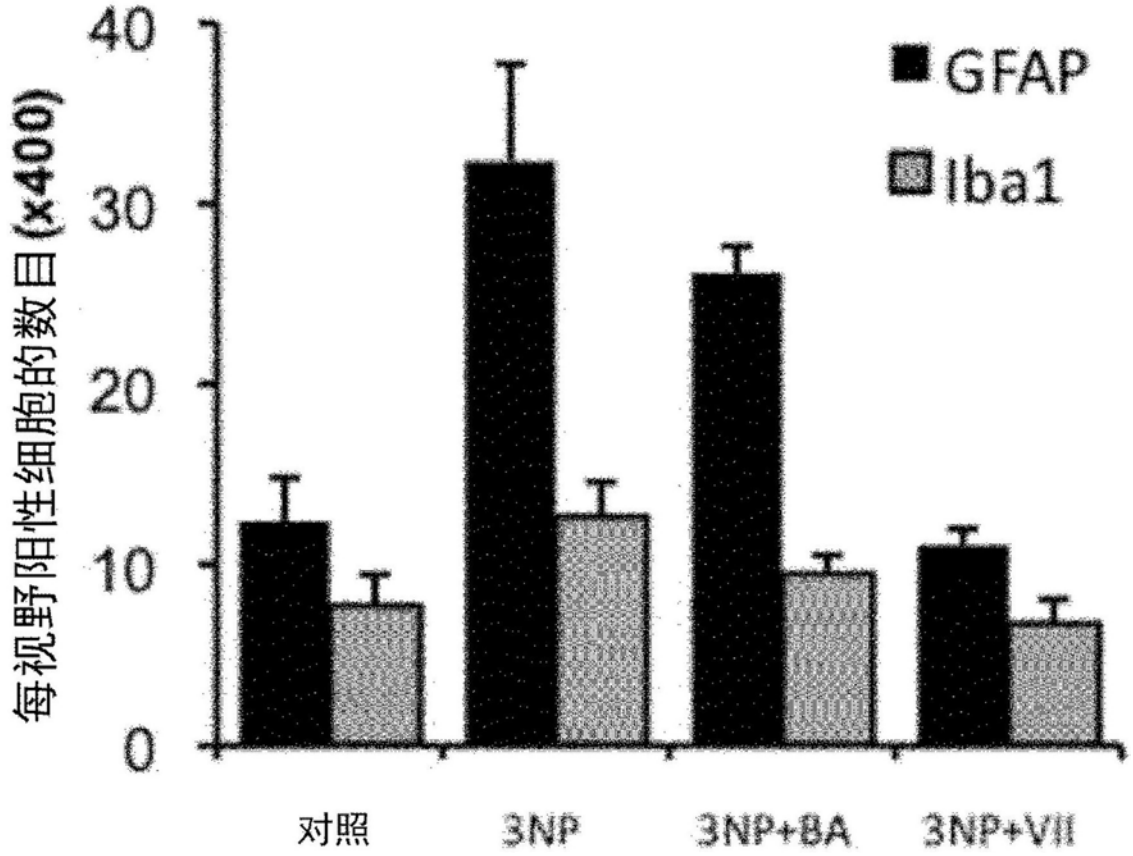
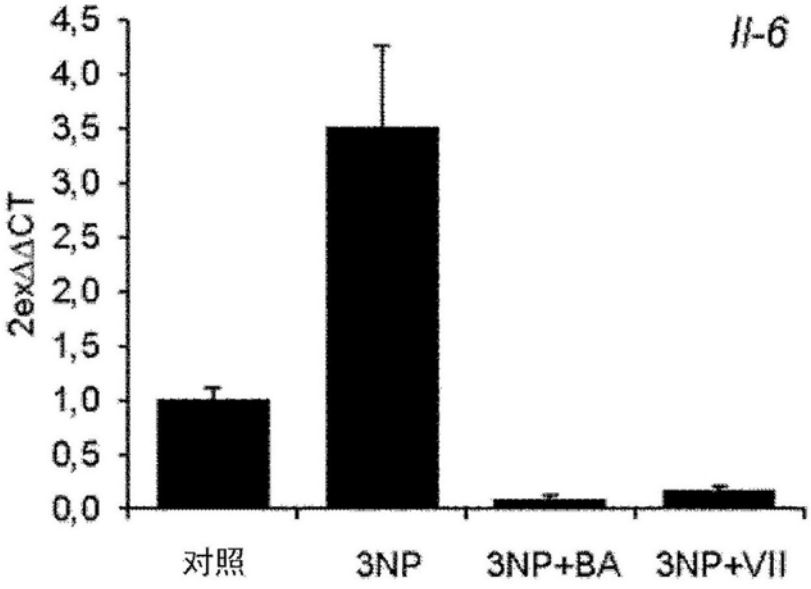


图11

12A


Il-6
2exΔΔCT
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
对照
3NP
3NP+BA
3NP+VII

12B

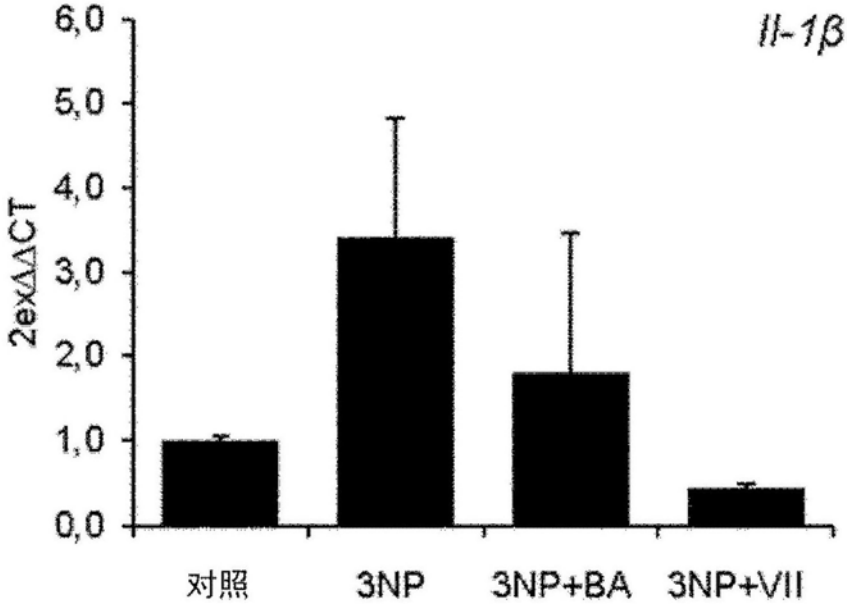
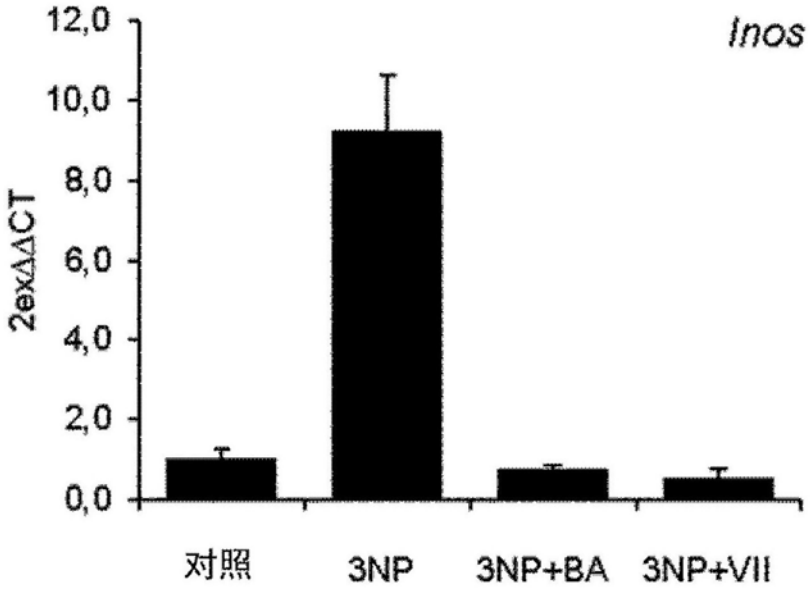

Il-1β
2exΔΔCT
6,0
5,0
4,0
3,0
2,0
1,0
0,0
对照
3NP
3NP+BA
3NP+VII

图12

12C


Inos
12,0
10,0
8,0
6,0
4,0
2,0
0,0
2exΔΔCT
对照
3NP
3NP+BA
3NP+VII

12D

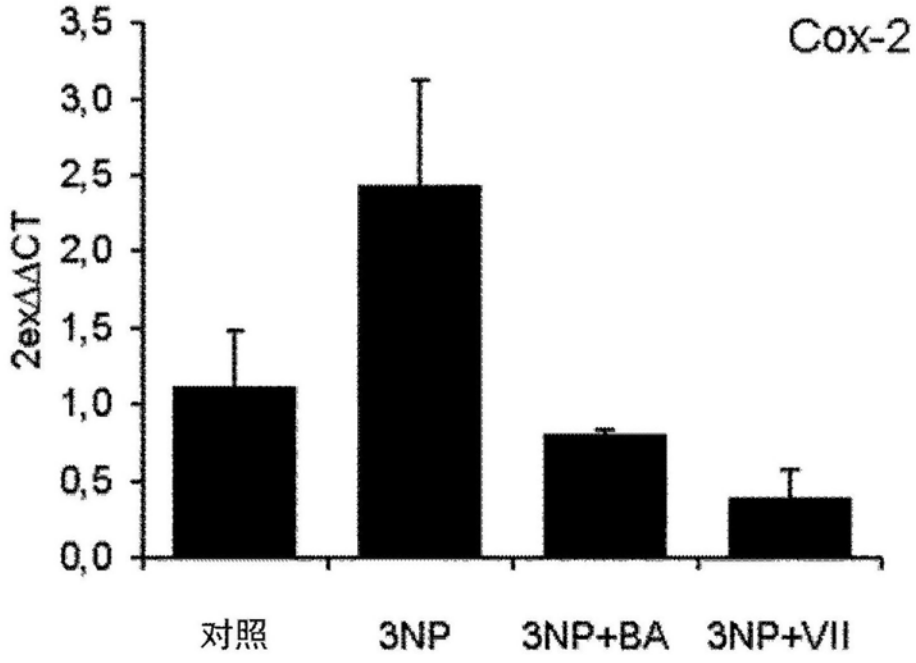
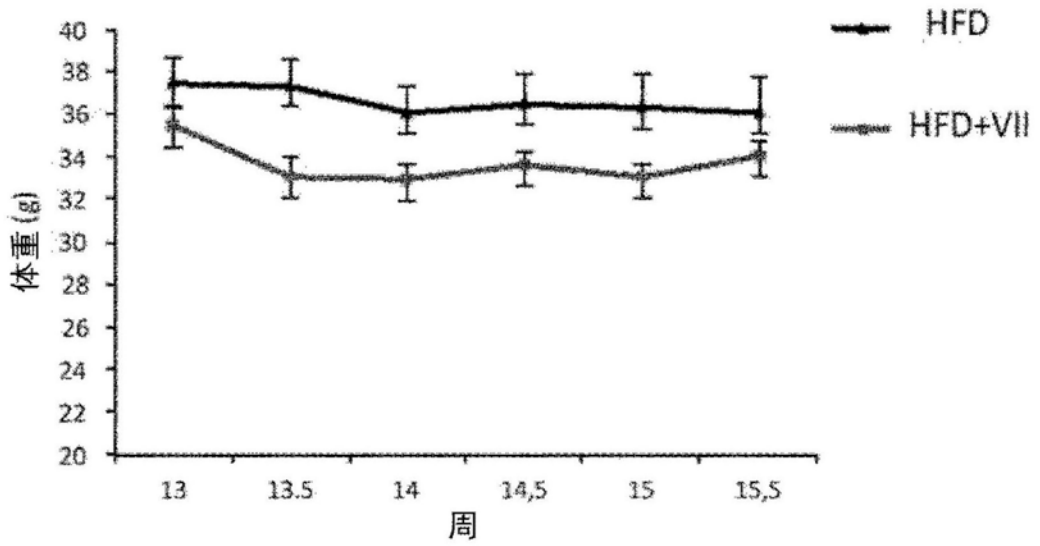
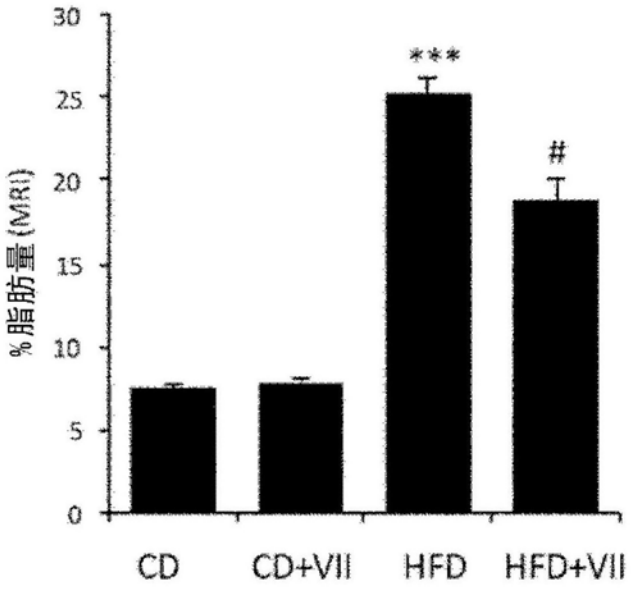

Cox-2
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
2exΔΔCT
对照
3NP
3NP+BA
3NP+VII

图12(续)

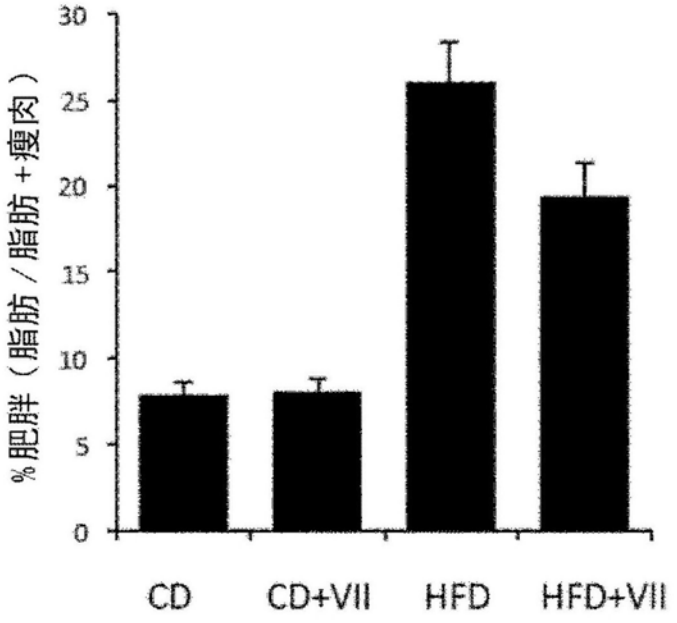

HFD
HFD+VII
体重(g)
40
38
36
34
32
30
28
26
24
22
20
13
13.5
14
14,5
15
15,5
周

13A


%脂肪量(MRI)
30
25
20
15
10
5
0
***
#
CD
CD+VII
HFD
HFD+VII

13B

图13


30
25
20
15
10
5
0
% 肥胖（脂肪 / 脂肪 + 瘦肉）
CD
CD+VII
HFD
HFD+VII

13C

图13(续)

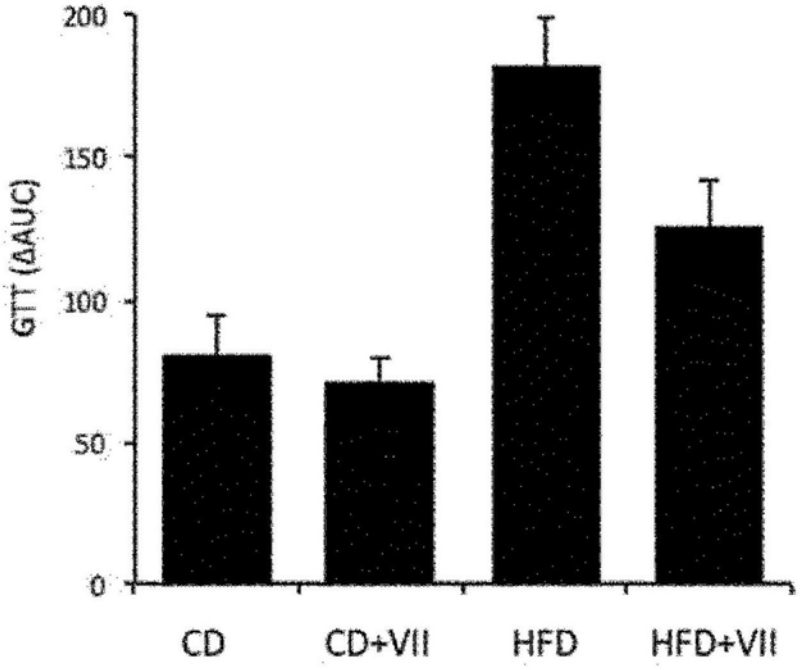

200
150
100
50
0
GTT (ΔAUC)
CD
CD+VII
HFD
HFD+VII

图14

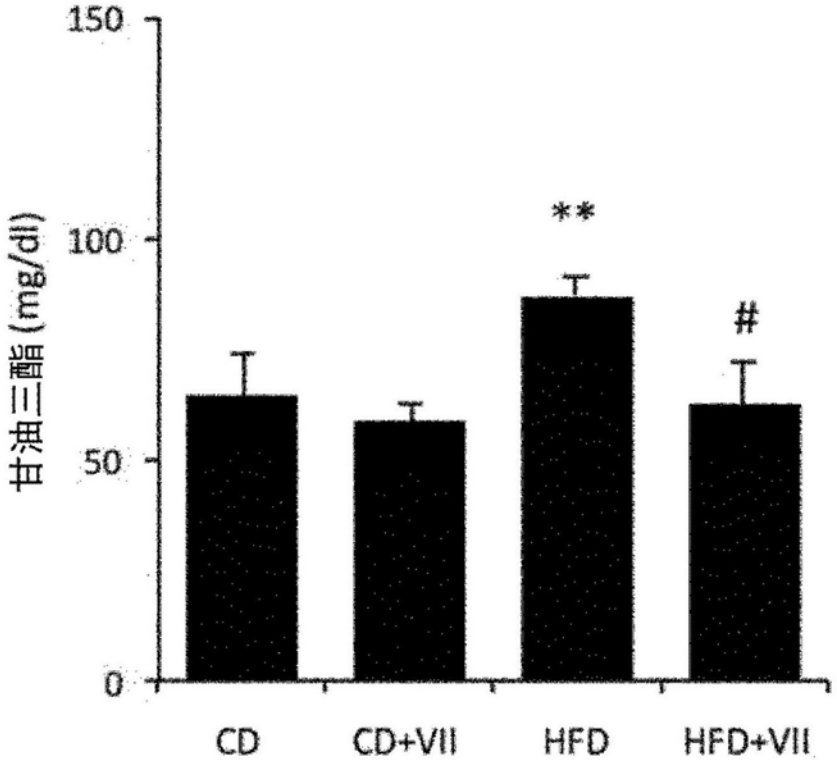


图15