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(54) Title: USE OF NITROOXYDERIVATIVE OF PARACETAMOL FOR THE TREATMENT OF MUSCULAR DYSTROPHIES

(57) Abstract: The present invention relates to the use of nitric oxide releasing derivatives of paracetamol for retarding or reversing muscular dystrophies such as Duchenne and Becker dystrophies.



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Use of nitrooxyderivative of paracetamol for the treatment of muscular dystrophies.

5

The present invention relates to the use of nitric oxide releasing compounds for retarding or reversing muscular dystrophies including Duchenne and Becker dystrophies.

10

Muscular dystrophies are caused by progressive degeneration of skeletal muscle fibres. Lack of one of several proteins located either at the plasma membrane or within internal membranes, increases the probability of damage during contraction, and eventually leads to fibre
15 degeneration, accompanied by severe local inflammation with infiltration of immune-competent cells.

Muscular dystrophies encompass a group of inherited, progressive muscle disorders, distinguished clinically by the selective distribution of skeletal muscle weakness. The
20 two most common forms of muscle dystrophy are Duchenne and Becker dystrophies, each resulting from the inheritance of a mutation in the dystrophin gene, which is located at the Xp21 locus. Other dystrophies include, but are not limited to, limb-girdle muscular dystrophy, fascioscapulohumeral
25 (Landouzy-Dejerine) muscular dystrophy, congenital muscular dystrophy, myotonic dystrophy, and Emery-Dreifuss muscular dystrophy.

In the most severe form, such as Duchenne muscular dystrophy, regeneration is exhausted and skeletal muscle is
30 progressively replaced by fat and fibrous tissue. This condition leads to patient showing a progressive weakness and eventually death by respiratory and/or cardiac failure.

The symptoms of Duchenne muscular dystrophy occur almost exclusively in males, and start at about 3-7 years of age with most patients confined to a wheelchair by 10-12 years and many die at about 20 years of age due to
5 respiratory complications.

Of the different medications that have been tried as potential treatments for Duchenne muscular dystrophy, only the corticosteroids such as prednisone, prednisolone and deflazacort have shown potential for providing temporary
10 improvement. This improvement results mainly from slowing the rate of progression or stabilizing muscle strength and function. Corticosteroid therapy also leads to side effects; as yet there is no consensus regarding their use as standard treatment.

15 Corticosteroids, such as prednisone, are believed to act by blocking the immune cell activation and infiltration which are precipitated by muscle fibre damage resulting from the disease.

The long-term therapies with corticosteroids for the
20 treatment of muscular dystrophy, are associated with adverse effects such as osteoporosis, hypertension and Cushing syndrome, weight gain, cataracts, short stature, gastrointestinal symptoms, behavioural changes in case of the prednisolone and weight gain and cataracts as for
25 deflazacort.

Bredt D. S. in Proc. Natl. Acad. Sci. USA 95 (1998), 14592-14593 reports that nitric oxide (NO) generated by muscular NO synthase, which is structurally and functionally linked to the dystrophin complex at the
30 sarcolemma, participates to physiological development and function of skeletal muscle by regulating vasodilation and thus supply oxygen during exercise, by increasing glucose

uptake in the myofibres and by regulating the enzymes activity relevant to cell energy metabolism.

EP 1252133 relates to nitrooxy derivatives of drugs to be used in oxidative stress and endothelial dysfunctions of moderate intensity. Nitrooxy derivatives of paracetamol are disclosed in particular.

EP 1339665 discloses the use of nitroderivatives of paracetamol, among other anti-inflammatory drugs, in the treatment of pre-cancer or cancer diseases on an inflammatory basis.

These documents do not report that these compounds are active for the treatment of muscular degenerative diseases.

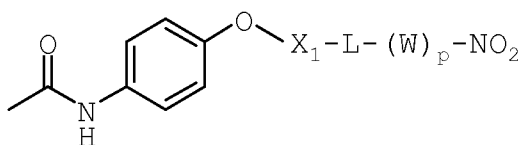
WO 00/53191 discloses the use of nitric oxide (NO), NO donors, inhibitors of NO activity or regulators of NO production, for the treatment of muscle diseases, which include Duchenne dystrophy, Becker dystrophy, limb-girdle muscular dystrophy, fascioscapulohumeral (Landouzy-Dejerine) muscular dystrophy, congenital muscular dystrophy, myotonic dystrophy, and Emery-Dreifuss muscular dystrophy. In particular the document discloses the results of a study of mdx dystrophic mice treated with deflazacort, deflazacort plus L-NAME (a NOS enzymes inhibitor) or deflazacort plus L-arginine (a NO donor). In the experiment, muscle tissues of tibialis anterior muscle and diaphragm were collected from the treated animal and the central nucleation index (CNI), which is a useful measure of muscle damage index, was assessed. The results show that the addition of the NO donor L-arginine to deflazacort did not improve the status of muscles in mdx mice, while L-NAME augmented the beneficial effects of deflazacort on diaphragm. The author concluded that the results show that L-NAME or other NOS inhibitors can be used for improving the effects of steroid when applied in situ.

WO 2007/088123 discloses the use of nitric oxide releasing derivatives of NSAIDs and statins for retarding or reversing muscular dystrophies. Said derivatives combine the known anti-inflammatory efficacy of NSAIDs and statins to the biological properties of NO, leading to a persistent amelioration of the dystrophic phenotype in the α -sarcoglycan null mouse model.

There is a need to identify therapeutic agents that slow the muscle fibres damage and delay the onset of disability in patients with muscular dystrophies, but cause a lesser degree of skeletal muscle atrophy than current therapies.

Quite surprisingly and unexpectedly, it was found that nitric oxide releasing derivatives of paracetamol, a known analgesic and antipyretic drug that does not show anti-inflammatory activity, are effective for retarding and reducing muscle damage and have long lasting therapeutic efficacy by slowing the process of muscular dystrophy. They have the advantage that they induce fewer adverse side effects, they are well tolerated by the patients and therefore they can be used in long term therapies. Moreover, paracetamol is commonly used in children; thus, this compound may be perspectively used in young patients.

It is an object of the present invention the use for the treatment of muscular dystrophies of (NO)-donor paracetamol derivatives of formula (I) and pharmaceutically acceptable salts or stereoisomers thereof:



(I)

wherein:

$X_1 = -(\text{CO})-\text{Y}-\text{T}_{\text{BI}}-$

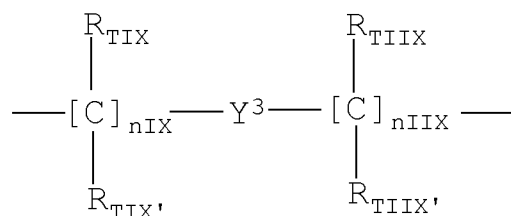
wherein

$\text{T}_{\text{BI}} = (\text{CO})$ or (X) wherein X is O , S , $\text{NR}_{1\text{C}}$, $\text{R}_{1\text{C}}$ is H or a linear or branched alkyl having from 1 to 5 carbon

5 atoms;

Y is a bivalent linking group selected from the following:

-



10

(II)

wherein:

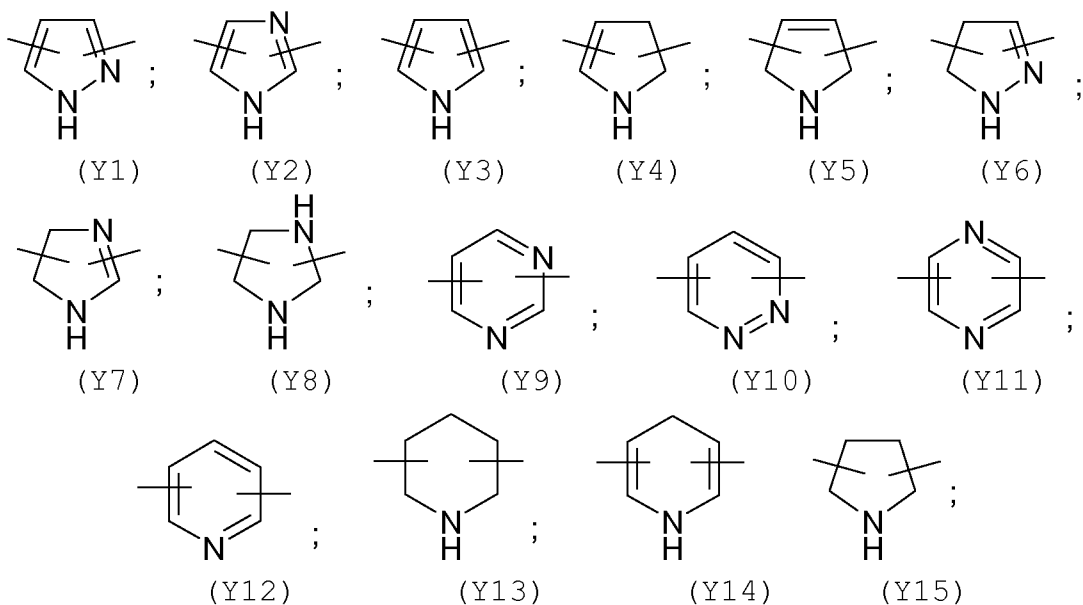
$n\text{IX}$ is an integer in the range 0-3;

$n\text{IIX}$ is an integer in the range 1-3;

R_{TIX} , $\text{R}_{\text{TIX}'}$, R_{TIIIX} , $\text{R}_{\text{TIIIX}'}$ are H ;

15

Y^3 is selected from:

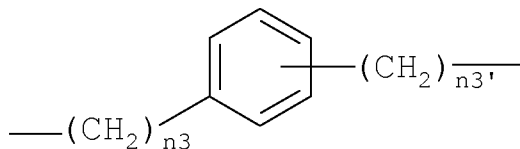


20

- an alkylene group R' wherein R' is a linear or branched $\text{C}_1\text{-C}_{20}$, optionally substituted with one or more

of the following groups -NHCOR_3 wherein R_3 is a linear or branched $\text{C}_1\text{-C}_5$ alkyl, -NH_2 or -OH ;

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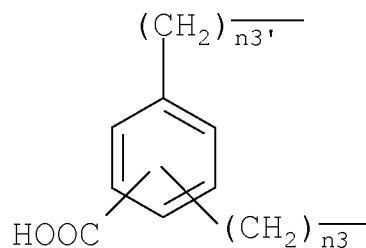
5

(III)

wherein n_3 is an integer from 0 to 3 and $n_{3'}$ is an integer from 1 to 3;

10

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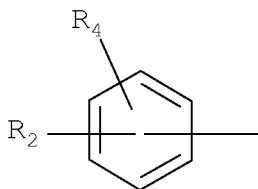


(IV)

15

wherein n_3 and $n_{3'}$ have the above reported meaning,

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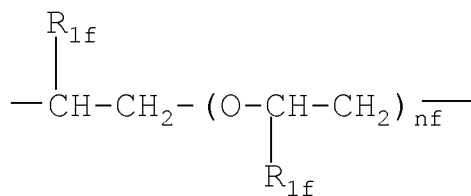
(V)

wherein

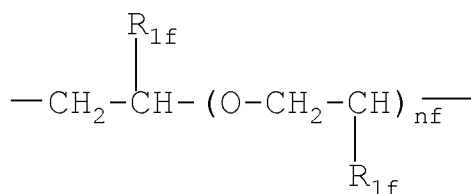
20

R_4 is hydroxy, hydrogen, $\text{R}_5\text{O-}$ wherein R_5 is a linear or branched $\text{C}_1\text{-C}_{10}$ alkyl group;

R_2 is a linear or branched $\text{C}_2\text{-C}_{10}$ alkenylene which can contain one or more double bonds;



(VI)



(VII)

wherein $\text{R}_{1f} = \text{H}, \text{CH}_3$ and nf is an integer from 0 to 6;

$\text{L} = \text{covalent bond}$ or $\text{L} = \text{X}$, X being as above defined, or

$\text{L} = \text{C}(\text{O})$;

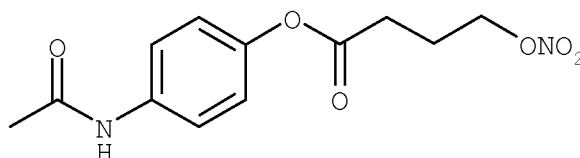
10 $\text{W} = \text{Y}_\text{T}\text{O}$ wherein Y_T has the same meanings of Y and Y_T in the compound of formula (I) is equal to or different from Y ;

p is an integer equal to 1 or 0.

15 Another preferred embodiment of the invention comprises the use of a nitric oxide-releasing drug of formula (I) for treatment of muscular dystrophies, wherein said drug is selected from the group consisting of:

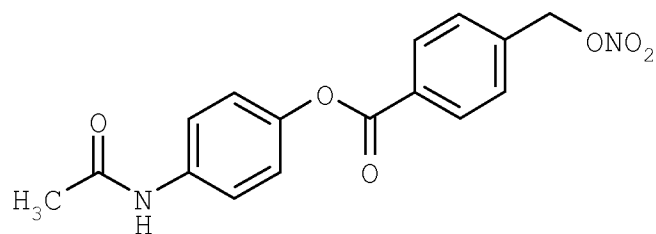
4-(nitrooxy)butanoic acid 4-(N-acetylamino)phenyl ester

20 (1),



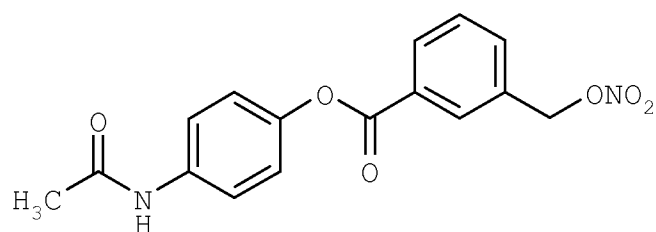
(1)

4-(nitrooxymethyl)-benzoic acid 4-(N-acetylamino)phenyl ester (2)



(2)

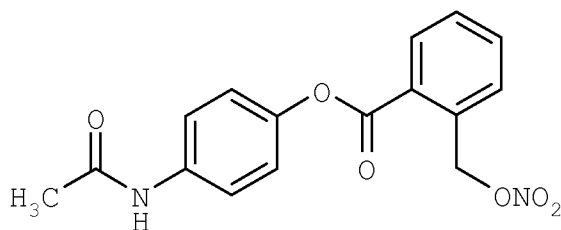
3-(nitrooxymethyl)-benzoic acid 4-(N-acetylamino)phenyl
ester (3)



(3)

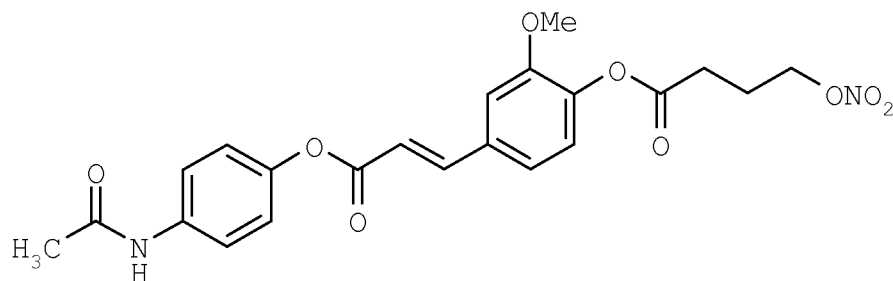
5

10 2-(nitrooxymethyl)-benzoic acid 4-(N-acetylamino)phenyl
ester (4)



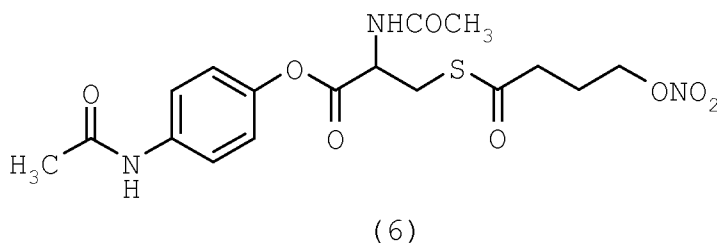
(4)

trans-3-[4-(4'-nitrooxybutyryloxy)-3-methoxyphenyl]-2-
15 propenoic acid 4-(N-acetylamino)phenyl ester (5)

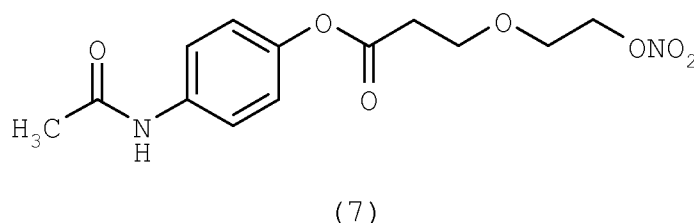


(5)

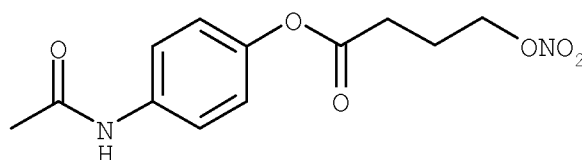
2-acetylamino-(4-nitrooxybutyryl)-3-mercaptopropionic acid
4-(N-acetylamino)phenyl ester (6)



5 3-[(2-nitrooxy)ethyloxy]propanoic acid 4-(N-acetylamino)phenyl ester (7)



10 A specific embodiment of the present invention comprises the use of the nitric oxide releasing drug 4-(nitrooxy)butanoic acid 4-(N-acetylamino)phenyl ester of formula (1),



15 for the treatment of muscular dystrophies.

20 The doses to be administered are determined depending upon, for example, age, body weight, symptom, the desired therapeutic effect, the route of administration, and the duration of the treatment. In the human adult, the doses per person at a time are generally from 1 mg to 1000 mg, by oral administration, up to several times per day, and from 1 mg to 100 mg, by parenteral administration (preferably

intravenous administration), up to several times per day, or continuous administration for from 1 to 24 hours.

As mentioned above, the doses to be used depend upon various conditions. Therefore, there are cases wherein
5 doses lower than or greater than the ranges specified above may be used.

The compounds of the present invention may be administered in the form of, for example, solid compositions, liquid compositions or other compositions for
10 oral administration, injections, liniments or suppositories for parenteral administration.

The nitric oxide releasing paracetamol derivatives as well as the methods for their preparation are disclosed in WO 02/30866.

15 Paracetamol is also known as acetaminophen.

Experimental part:

20 Animal model: α -sarcoglycan null mouse (α -SG null C57BL/6 x 129/Sv mouse): this is a well-known model of muscle dystrophies (Brunelli et al., PNAS, 2007; 104(1): 264-269). Time frame: 1-9 months.

Animals were treated with the drugs starting at 1.5 months
25 of age.

The tested compounds are:

- 4-(nitrooxy)butanoic acid 4-(N-acetylamino)phenyl ester
- paracetamol (para) as reference

30 To assess muscle function and structure, creatine kinase blood concentrations and resistance to fatigue were evaluated. Creatine kinase, an enzyme released by damaged or necrotic fibres, is commonly used as biomarker of the

severity of muscular dystrophies. Muscle function was evaluated by using the running-wheel and the treadmill tests, which measure animal voluntary motility and muscle endurance, respectively.

- 5 α -SG null mice were treated with the diet containing 4-(nitrooxy)butanoic acid 4-(N-acetylamino)phenyl ester (187 mg/kg/die) to test the efficacy as a therapeutic treatment for muscular dystrophy.
- 10 Untreated controls (NT) and animals receiving only paracetamol (para) (100 mg/kg/die, administered daily in the diet starting at 1 month from weaning for up to 9 months) had been set in parallel as controls.

Experimental tests:

- 15 - Creatine kinase activity measurements: quantitative and kinetic determination of creatine kinase activity in serum of control and drug treated-animals was measured using creatine kinase reagent (Sigma colorimetric assay), according to the manufacturer's
- 20 instructions. Blood was collected from the tail vein and serum obtained after centrifugation at 13,000 rpm for 10 minutes was stored at -80°C before measurements. The data are reported in Table I. Values shown are the result of experiments on 3 animals per
- 25 group $\pm$ SEM.
- Locomotor activity (Mobility):
- Running-wheel activity:* voluntary wheel running was used as the exercise paradigm to avoid any physiological changes that may occur due to the stress
- 30 of forced treadmill running. Mice were housed individually in cages (9 x 22 x 9 cm check) equipped with a running wheel (20-cm in diameter; Trixie, Tarp, Germany). Each wheel revolution was registered through

a magnetic switch, which was connected to a counter. The number of revolutions was recorded daily for 6 days.

5 The free wheel values, reported as distance in km covered in 24 hours, were measured after 5 months of treatment of α -SG null mice. Values shown are the result of experiments on 3 animals per group $\pm$ SEM (Table II).

10 *Exhaustion treadmill:* the analyses were carried out using a six-lane motorized treadmill (Exer 3/6 Treadmill; Columbus Instruments, Columbus, OH) supplied with shocker plates. The treadmill was run at an inclination of 0° at 12 m/min for 30 minutes. The test was stopped when the mouse remained on the
15 shocker plate for more than 20 sec without attempting to re-engage the treadmill. The time to exhaustion was determined from the beginning of the test. Three tests were performed at the 7th month on the same animal, 7 days after creatine kinase assay. Values obtained were
20 averaged, providing a single value per animal. Values shown are the result of experiments on 3 animals per group $\pm$ SEM (Table II).

The results show that the tested compound (1) was
25 effective in reducing the functional and biochemical alterations, which typically occur in these animals. In particular, the tested compound (1) was more effective in reducing the plasma levels of creatine kinase, a hallmark of muscle damage, in treated animals (Table I) vs both the
30 untreated control (control) and paracetamol (para); consistently, treated animals performed significantly better on both free-wheel running test and treadmill test (Table II).

Altogether, the data show that the compounds of the present invention have a better profile as compared to paracetamol.

5

Table I:

Compound	Months of treat.	Creatine kinase blood concentrations (UI/ml $\pm$ SEM)
NT	1	546.9 $\pm$ 61.0
	2	1260.9 $\pm$ 140.2
	3	1312.0 $\pm$ 255.0
	4	832.0 $\pm$ 106.0
	6	255.9 $\pm$ 32.5
Para	1	456.0 $\pm$ 60.0
	2	915.0 $\pm$ 136.0
	3	984.6 $\pm$ 150.0
	4	1122.0 $\pm$ 97.0*
	6	254.5 $\pm$ 33.0
(1)	1	359.8 $\pm$ 76.1
	2	880.8 $\pm$ 168.0*
	3	897.4 $\pm$ 99.0*
	4	766.0 $\pm$ 65.0
	6	234.0 $\pm$ 46.0

* $p < 0.05$ vs NT

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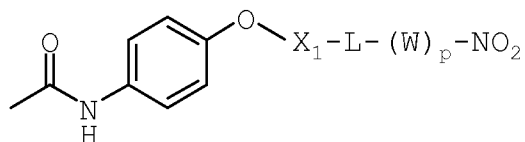
Table II:

Compound	Locomotor activity	
	Free wheel running distance, km $\pm$ SEM	Treadmill time of exhaustion, min $\pm$ SEM
NT	0.9 $\pm$ 0.1	21.6 $\pm$ 3.9
Para	1.9 $\pm$ 0.6	32.8 $\pm$ 9.1*
(1)	2.6 $\pm$ 0.6*	38.3 $\pm$ 5.5*

* p< 0.05 vs NT

CLAIMS

1. A nitric oxide releasing compound of formula (I)



5 (I)

or pharmaceutically acceptable salts or stereoisomers thereof, wherein in the general formula (I) X_1 , L, W and p and Y have the following meanings:

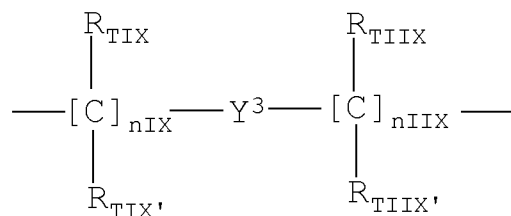
$X_1 = -(\text{CO})-\text{Y}-\text{T}_{\text{BI}}-$

10 wherein

$\text{T}_{\text{BI}} = (\text{CO})$ or (X) wherein X is O, S, $\text{NR}_{1\text{C}}$, $\text{R}_{1\text{C}}$ is H or a linear or branched alkyl having from 1 to 5 carbon atoms;

Y is a bivalent linking group selected from the following:

-



(II)

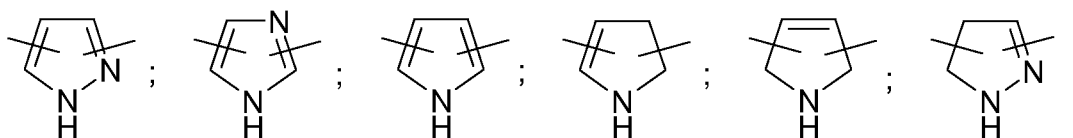
wherein:

20 n_{IX} is an integer in the range 0-3;

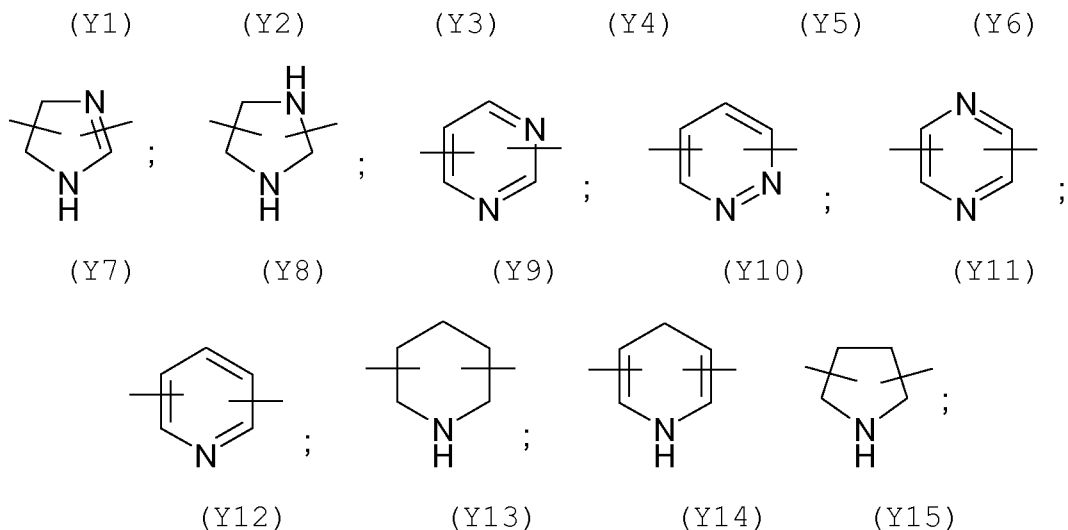
n_{IIX} is an integer in the range 1-3;

R_{TIX} , $\text{R}_{\text{TIX}'}$, R_{TIIX} , $\text{R}_{\text{TIIX}'}$ are H;

Y^3 is selected from:



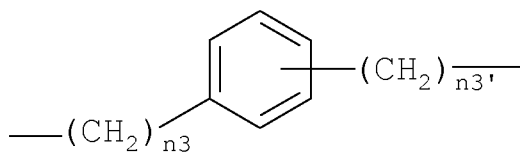
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5

- an alkylene group R' wherein R' is a linear or branched C_1 - C_{20} , optionally substituted with one or more of the following groups $-NHCOR_3$ wherein R_3 is a linear or branched C_1 - C_5 alkyl, $-NH_2$ or $-OH$;

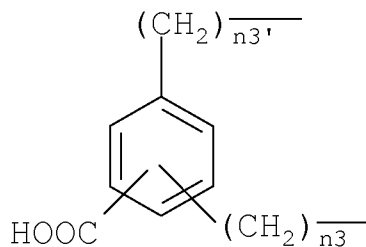
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(III)

wherein n_3 is an integer from 0 to 3 and $n_{3'}$ is an integer from 1 to 3;

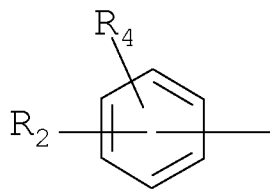
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(IV)

wherein n_3 and $n_{3'}$ have the above reported meaning,

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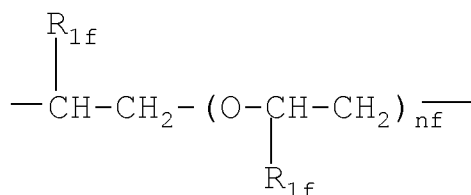
(V)

wherein

5 R_4 is hydroxy, hydrogen, R_5O- wherein R_5 is a linear or branched C_1-C_{10} alkyl group;

R_2 is a linear or branched C_2-C_{10} alkenylene which can contain one or more double bonds;

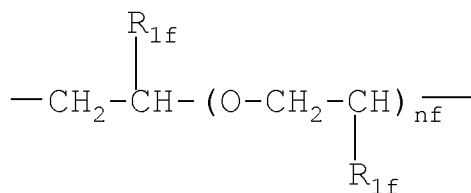
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(VI)

10

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(VII)

wherein $R_{1f} = H, CH_3$ and nf is an integer from 0 to 6;

15 $L =$ covalent bond or $L = X$, X being as above defined, or $L = C(O)$;

$W = Y_T O$ wherein Y_T has the same meanings of Y and Y_T in the compound of formula (I) is equal to or different from Y ;

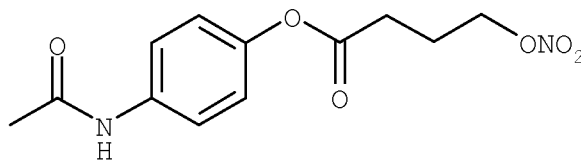
20 p is an integer equal to 1 or 0

for use in the treatment of muscular dystrophies.

2. A compound of formula (I) selected from the group consisting of:

4-(nitrooxy)butanoic acid 4-(N-acetylamino)phenyl ester (1),

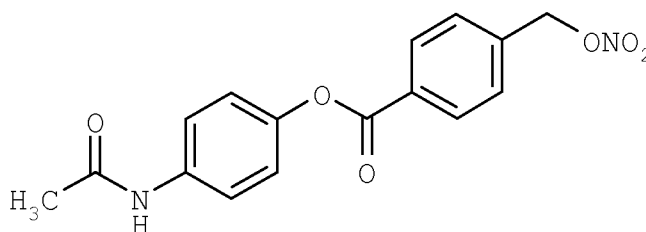
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(1)

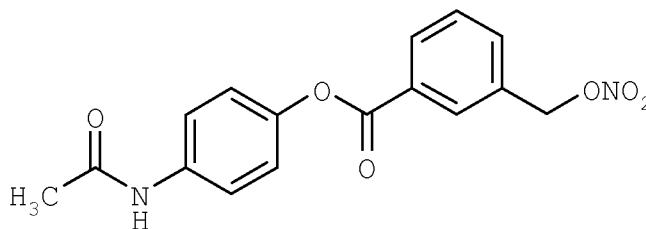
4-(nitrooxymethyl)-benzoic acid 4-(N-acetylamino)phenyl ester (2)

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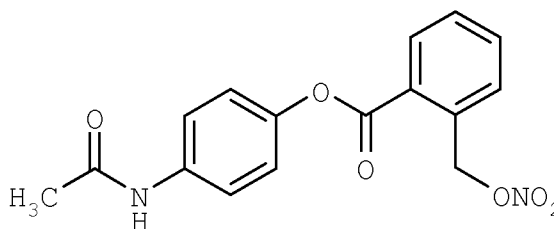
(2)

3-(nitrooxymethyl)-benzoic acid 4-(N-acetylamino)phenyl ester (3)



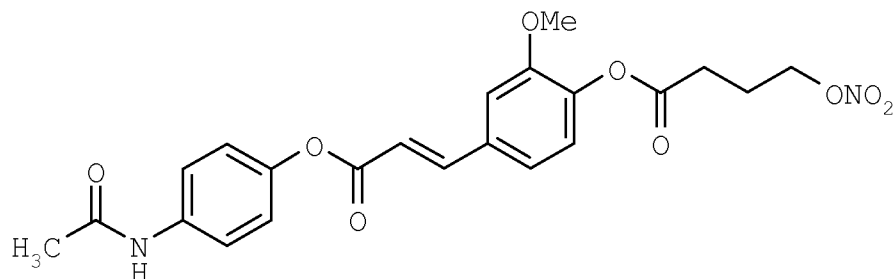
(3)

15 2-(nitrooxymethyl)-benzoic acid 4-(N-acetylamino)phenyl ester (4)



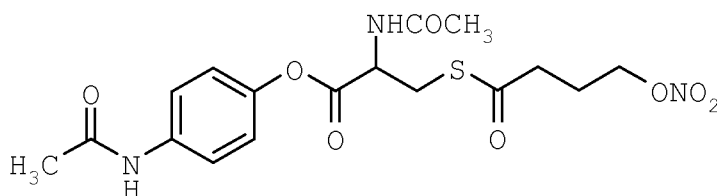
(4)

trans-3-[4-(4'-nitrooxybutyryloxy)-3-methoxyphenyl]-2-propenoic acid 4-(N-acetylamino)phenyl ester (5)



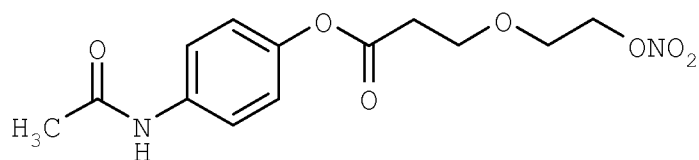
(5)

- 5 2-acetylamino-(4-nitrooxybutyryl)-3-mercaptopropionic acid 4-(N-acetylamino)phenyl ester (6)



(6)

- 10 3-[(2-nitrooxy)ethyloxy]propanoic acid 4-(N-acetylamino)phenyl ester (7)

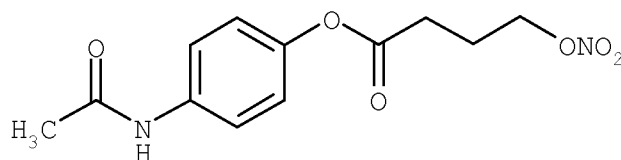


(7)

for use in the treatment of muscular dystrophies.

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3. A compound of formula (I) which is 4-(nitrooxy)butanoic acid 4-(N-acetylamino)phenyl ester of formula (1)



(1)

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for use in the treatment of muscular dystrophies.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/053521

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/167 A61P21/00
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)

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 practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCIORATI C ET AL: "Therapeutic efficacy and mechanism of action of nitric oxide-releasing non steroidal anti-inflammatory drugs in muscular dystrophy" INTERNET CITATION 1 January 2008 (2008-01-01), page 1PP, XP009133106 Retrieved from the Internet: URL: http://www.myology2008.org/assets/files/PDF/PW%2025.pdf [retrieved on 2010-05-04]	1-3
Y	the whole document	1-3
Y	WO 2007/088123 A2 (NICOX SA [FR]; CLEMENTI EMILIO [IT]; COSSU GIULIO [IT]; BRUNELLI SILVI) 9 August 2007 (2007-08-09) claims 1-5	1-3
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Further documents are listed in the continuation of Box C.



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 document but published on or after the international filing date

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"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

18 May 2010

Date of mailing of the international search report

25/05/2010

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/053521

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BRUNELLI SILVIA ET AL: "Nitric oxide release combined with nonsteroidal anti inflammatory activity prevents muscular dystrophy pathology and enhances stem cell therapy" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES (PNAS), NATIONAL ACADEMY OF SCIENCE, US LNKD-DOI:10.1073/PNAS.0608277104, vol. 104, no. 1, 2 January 2007 (2007-01-02), pages 264-269, XP002445669 ISSN: 0027-8424 abstract	1-3
Y	US 2008/194651 A1 (DEL SOLDATO PIERO [IT] ET AL) 14 August 2008 (2008-08-14) abstract page 10, paragraph 210 - page 13, paragraph 255; examples 8-14	1-3
Y	WO 2005/054175 A2 (NICOX SA [FR]; RIVOLTA ROMANO [FR]; AURELI ROBERTO [IT]) 16 June 2005 (2005-06-16) abstract page 1, line 5 - line 12 page 8 claim 1	1-3
Y,P	WO 2009/087005 A2 (NICOX SA [FR]; IMPAGNATIELLO FRANCESCO [IT]; HERRERO JUAN F [ES]; BENE) 16 July 2009 (2009-07-16) claims	1-3

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Information on patent family members

International application No

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