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(54) Title: USE OF CYCLOUNDECADEPSIPEPTIDE COMPOUNDS

(57) Abstract: The present invention relates to cycloundecadepsipeptide compound analogues presenting mitochondrial permeability transition pore (MPTP) inhibitory activity and reduced cytotoxicity. The present invention further relates to a pharmaceutical composition containing cycloundecadepsipeptide compound and analogues for use in the treatment or prevention of cell death associated disorders or diseases, related to the MPTP opening.

Use of cycloundecadepsipeptide compounds

Field of the invention

The present invention relates to cycloundecadepsipeptide compounds and analogues presenting mitochondrial permeability transition pore (MPTP) inhibitory activity and reduced cytotoxicity. The present invention further relates to the pharmaceutical compositions containing cycloundecadepsipeptide compounds and analogues for use in the treatment and/or prevention of cell death associated disorders and diseases, related to the MPTP opening.

Background of the invention

In addition to their central role in ATP synthesis, mitochondria play a critical role in cell death. Oxidative stress accompanied by calcium overload, ATP depletion, and elevated phosphate levels induces mitochondrial permeability transition (MPT) with formation of nonspecific MPT pores (MPTP) in the inner mitochondrial membrane. Mitochondrial permeability transition (MPT) is an increase in the permeability of the mitochondrial membranes to molecules of less than 1500 Daltons in molecular weight. MPT results from opening of mitochondrial permeability transition pores (MPT pores or MPTP) (Halestrap 2010).

MPT induction is a key event in cell death and is implicated in a variety of disorders and diseases. For example:

- It is involved in cell death in excitotoxicity (Abramov and Duchen 2008; Malouitre, Dube et al. 2009), in which overactivation of glutamate receptors causes excessive calcium entry into the cell.
- MPT also appears to play a key role in damage caused by ischemia, as occurs in a heart attack and stroke (Baines, Kaiser et al. 2005; Nakagawa, Shimizu et al. 2005; Schinzel, Takeuchi et al. 2005; Halestrap 2010).

- Research has shown that the MPT pore remains closed during ischemia, but opens once the tissues are reperfused with blood after the ischemic period, playing a role in reperfusion injury (Lim, Hausenloy et al. 2010).
- MPT is also thought to underlie the cell death induced by Reye's syndrome, since chemicals that can cause the syndrome, like salicylate and valproate, cause MPT (Trost and Lemasters 1996).
- MPT may also play a role in mitochondrial autophagy (Lemasters, Nieminen et al. 1998).
- Cells exposed to toxic amounts of Ca^{2+} ionophores also undergo MPT and death by necrosis (Tazawa, Fujita et al. 2009).
- Neurodegeneration, a process that results in damage and death of neurons, is also a disorder where MPTP is found to be involved (Barrientos, Martinez et al. ; Du and Yan ; Du, Guo et al. 2008; Ibarra and Martinon 2009; Martin, Gertz et al. 2009).
- It has been shown that MPTP plays an important role in muscular dystrophies (*Palma, E., T. Tiepolo, et al. 2009; Millay, D. P., M. A. Sargent, et al. 2008*
- MPTP is involved in cell death induced by metabolic stress and/or oxidative stress and/or loss of cellular calcium homeostasis and/or aging cells and/or toxic agents (pathogens) and/or infectious agents (pathogens) *(Burke et al. 2007; King et al. 2008; Sokol et al. 2005; Plotnikov et al. 2009).*

Accordingly, MPTP is accepted as a therapeutic target for both pharmacological and conditional strategies to block pore formation by direct interaction with MPTP components or indirectly by decreasing MPTP inducers (Zorov, Juhaszova et al. 2009; Giorgio, Soriano et al. 2010). Inhibition of MPTP opening by reduction of CyP-D activity by nonimmunosuppressive analogs of cyclosporine A or sanglifehrin A, as well as attenuation of reactive oxygen species accumulation through mitochondria-targeted antioxidants, is the most promising.

Inhibiting the opening of the MPTP can treat and/or prevent cell death associated disorders and diseases. As documented above, it is known that the MPTP opening has an important role in triggering organ dysfunction and pathology. Cell death associated disorders and diseases, wherein the MPTP opening is involved,

include but are not limited to, ischaemia-reperfusion injuries, metabolic and/or oxidative stress disorders, diseases involving loss of cellular calcium homeostasis, age related cellular degeneration, diseases involving toxic and/or infectious pathogens.

Ischaemia and ischaemia-reperfusion injury: the opening of the MPTP may be induced by ischaemia-reperfusion and can play an important role in the following diseases or conditions:

- acute cerebral ischemia (Schinzel, Takeuchi et al. 2005), acute stroke (Halestrap 2010),
- acute myocardial infarction cardiac arrest, myocardial stunning, post-reperfusion arrhythmias, organ failure following therapeutic procedures such as thrombolytic therapy, coronary angioplasty, aortic cross-clamping or coronary bypass surgery (Baines, Kaiser et al. 2005; Nakagawa, Shimizu et al. 2005; Halestrap 2010)
- hypovolemic shock, storage/reperfusion injury of transplant organs (Raisky, Gomez et al. 2004), multi organ dysfunction syndrome.

Other cell death associated diseases or disorders, related to the MPTP opening, involve organ dysfunction or pathologies in which the opening of the MPTP may be also an important trigger for cell death and is induced by metabolic stress and/or oxidative stress and/or loss of cellular calcium homeostasis and/or aging cells and/or toxic agents (pathogens) and/or infectious agents (pathogens) including but not limited to:

<u>Muscular dysthrophies</u>

- muscular dysthrophies, including but not limited to Ulrich congenital muscular dysthrophy, Bethlem myopathy, myosclerosis, limb girdle muscular dysthropy, Duchenne muscular dysthrophy, Becker muscular dysthrophy, Emery-Dreifuss syndrome (Angelin, Tiepolo et al. 2007; Millay, Sargent et al. 2008; Palma, Tiepolo et al. 2009; Tiepolo, Angelin et al. 2009; Wissing, Millay et al. 2010),

Neurodegeneration:

- traumatic brain injury (Mazzeo, Beat et al. 2009), spinal cord injury (Ravikumar, McEwen et al. 2007), or peripheral nerve injury (Barrientos, Martinez et al.), epilepsy-induced brain injury
- neurodegenerative diseases including but not limited to: amyothrophic lateral sclerosis (ALS) (Martin, Gertz et al. 2009; Martin 2010), Alzheimer disease (Du and Yan 2010), Parkinson disease , multiple sclerosis (Forte, Gold et al. 2007), Huntington disease (Brustovetsky, Brustovetsky et al. 2003)
- axonal degeneration-induced neuropathic pain, diabetic neuropathy chemotherapy-induced neuropathic pain, or herpes-induced neuropathy (Barrientos, Martinez et al.)

Cell death induced by metabolic stress and/or oxidative stress and/or loss of cellular calcium homeostasis and/or age related cellular degeneration and/or diseases involving toxic agents and/or infectious pathogens:

- West Nile viral encephalitis (Morrey, Siddharthan et al. 2010), Japanese encephalitis(Tsao et al. J Gen Virol. 2008 Aug;89(Pt 8):1930-41)
- Chronic hepatitis and cirrhosis induced by Hepatitis C virus (Piccoli, Scrima et al. 2007), Hepatitis B (Xia, Shen et al. 2005), delta agent,
- acute and chronic, drug- or toxin-induced hepatotoxicity (Soriano, Nicolosi et al. 2004; Masubuchi, Kano et al. 2006; Burke, Redeker et al. 2007), alcoholic liver disease (King, Swain et al. 2010; Shulga and Pastorino 2010), cholestasis, Non Alcoholic Fatty Liver Disease (Teodoro, Rolo et al. 2008), gastro-intestinal ulcerations caused by NSAIDs (LoGuidice, Ramirez-Alcantra et al. Toxicol Sci. 2010),
- transplant rejection (Raisky, Gomez et al. 2004; Gomez, Raisky et al. 2006),
- peripheral vascular insufficiency, renal insufficiency,
- obesity (Devalaraja-Narashimha, Diener et al. 2011), diabetic micro-angiopathy, diabetic nephropathy (Sun, Xiao et al. 2010), Maturity onset diabetes of the young type 4 (Fujimoto, Ford et al. 2010)

- acute and chronic pancreatitis (Mukherjee, Criddle et al. 2008),
- septicaemia (Larche, Lancel et al. 2006), multi organ dysfunction syndrome, crush syndrome,
- congestive heart failure (Halestrap 2010), atherosclerosis (Di Lisa and Bernardi 2009), hypertensive heart disease (Shabaz et al J Hypertens. 201 Sep; 28 Suppl 1 S25-32),
- cancer (Eliseev, Malecki et al. 2009)
- bipolar disorder (Kubota, Kasahara et al. 2010),

Agents that block MPT include the immune suppressant cyclosporin A (CsA); N-methyl-Val-4-cyclosporin A (MeVal-4-CsA), a non-immunosuppressant derivative of CsA; another non-immunosuppressive agent, NIM811, 2-aminoethoxydiphenyl borate (2-APB), and bongkrekic acid.

For example WO 2006/072639 (DEBIOPHARM SA) relates to the use of a cyclic undecapeptide for the preparation of a medicament for administration during a myocardial ischaemic event. Besides, WO 2009/098533 (DEBIOPHARM SA) relates to the use of a non-immunosuppressive cyclosporin A derivative for preventing or reducing muscular degeneration in a subject suffering from Limb-Girdle muscular Dystrophy.

Naturally occurring cyclosporins and derivatives present MPTP inhibitory activity but simultaneously display cytotoxicity which limits their anti-cell death effect. It is know that cyclosporins are able to inhibit MPTP activity via their binding to mitochondrial cyclophilin and that MPTP modulation is involved in many pathological processes. It is also known that cyclosporins display cytotoxicity.

Despite beneficial MPTP inhibition activity, the cyclosporins and derivatives display cytotoxicity which prevent their usage at higher doses and thus limit their therapeutic index. Overall, the anti-cell death effect of available cyclosporins and derivatives is limited by cytotoxicity. Thus there is a real need in obtaining compounds acting on MPTP and having reduced cytotoxicity which would result in an enhanced anti-cell death activity.

Summary of the invention

Therefore the aim of the present invention is to provide new series of compounds with reduced cytotoxicity resulting in enhanced anti-cell death activity.

The present invention provides a cycloundecadepsipeptide compound of Formula (I)

Cyclo-(AXX_1-AXX_2-AXX_3-AXX_4-AXX_5-AXX_6-AXX_7-D-Hiv-MeLeu-Leu-MeVal) (I)
1 2 3 4 5 6 7 8 9 10 11

in which:

AXX_1 is MeBmt, 4-fluoro-MeBmt, dihydro-MeBmt, 8-hydroxy-MeBmt; O-acetyl-MeBmt;

AXX_2 is Abu, Val, Thr, Thr(OMe), Thr(OAc), Thr($OCOCH_2CH_2CH_2OH$), Nva, 5-hydroxy-Nva (Hnv);

AXX_3 is D-MeAla, D-3-fluoro-MeAla, D-MeSer, D-MeSer(OAc), D-MeSer(OCH_2CH_2OH), D-MeSer($OCH_2CH_2NEt_2$), D-MeAsp(OMe);

AXX_4 is MeIle, MeMet, MeVal, MeThr, MeThr(OAc), MeAla, EtVal, EtIle, EtPhe, EtTyr, EtThr(OAc), MeThr(OAc), MeTyr, MeTyr(OAc), MeTyr(OMe), MePhe, MeMet(Ox) wherein the sulphur atom of methionine is sulphoxyde or sulphone;

AXX_5 is Leu, Val, Ile, Gly, Abu;

AXX_6 is MeAla, Sar, MeLeu; and

AXX_7 is Gly, Ala;

for use in a method for treating and/or preventing cell death associated disorders and diseases, related to the MPTP opening,

and wherein said cycloundecadepsipeptide compound of Formula (I) comply with the two following criteria:

(1) MPTP inhibitory activity at least equal to half of the CsA activity, defined as:

$$\frac{Compound\_of\_formula(I)\_CRC\_IC_2}{CsA\_CRC\_IC_2} \leq 2$$

(2) at least three-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 3$$

The present invention further provides the use of a pharmaceutical composition for treating and/or preventing cell death associated disorders and diseases, related to the MPTP opening, comprising a compound of the invention, together with one or more pharmaceutically acceptable diluents or carriers.

Brief description of figures

Figure 1 shows plasma amylase, interleukin-6, pancreatic myeloperoxidase and histopathology scores following treatment in the bile acid-induced murine acute pancreatitis model (Student's t test: * = $p < 0.05$ comparing compound 057 with vehicle).

Detailed description of the invention

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

As used herein, the following definitions are supplied in order to facilitate the understanding of the present invention.

The term “comprise” is generally used in the sense of include, that is to say permitting the presence of one or more features or components.

“Treatment” refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder or the disease as well as those in which the disorder is to be prevented. Hence, the mammal to be treated herein may have been diagnosed as having the disorder or the disease may be predisposed or susceptible to the disorder or the disease.

“Mammal” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals or pet animals, such as dogs, horses, cats, cows, monkeys etc. Preferably, the mammal is human.

The term “therapeutically effective amount” refers to an amount of a drug effective to treat a disease or disorder in a mammal.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

As used herein, the term “cell death associated disorders and diseases” comprises disorders and diseases caused by, associated with, linked with, mediated by or accompanied by the MPTP opening or wherein the MPTP opening is involved.

“Cell death” encompasses the normal degeneration and death of living cells, also defined as the terminal failure of a cell to maintain essential life functions, or the point in the process of dying at which vital functions have ceased at the cellular level. Cell death includes for example apoptosis as well as necrosis often defined as the morphological changes indicative of cell death caused by progressive enzymatic degradation; it may affect groups of cells or part of a structure or an organ.

As referred to herein a "disorder" refers to an underlying pathological disturbance in a symptomatic or asymptomatic organism relative to a normal organism, which may result, for example, from infection, stress or an acquired or congenital genetic imperfection.

The invention refers to a series of cycloundecadepsipeptide compounds, with or without immunosuppressant activity, presenting mitochondrial permeability transition pore (MPTP) inhibitory activity and reduced cytotoxicity.

It is an object of the present invention to provide for a cycloundecadepsipeptide compound of Formula (I)

Cyclo-(AXX_1-AXX_2-AXX_3-AXX_4-AXX_5-AXX_6-AXX_7-D-Hiv-MeLeu-Leu-MeVal) (I)

1 2 3 4 5 6 7 8 9 10 11

in which:

AXX_1 is MeBmt, 4-fluoro-MeBmt, dihydro-MeBmt, 8-hydroxy-MeBmt; O-acetyl-MeBmt;

AXX_2 is Abu, Val, Thr, Thr(OMe), Thr(OAc), Thr($OCOCH_2CH_2CH_2OH$), Nva, 5-hydroxy-Nva;

AXX_3 is D-MeAla, D-3-fluoro-MeAla, D-MeSer, D-MeSer(OAc), D-MeSer(OCH_2CH_2OH), D-MeSer($OCH_2CH_2NEt_2$), D-MeAsp(OMe);

AXX_4 is MeIle, MeMet, MeVal, MeThr, MeThr(OAc), MeAla, EtVal, EtIle, EtPhe, EtTyr, EtThr(OAc), MeThr(OAc), MeTyr, MeTyr(OAc), MeTyr(OMe), MePhe, MeMet(Ox) wherein the sulphur atom of methionine is sulphoxyde or sulphone;

AXX_5 is Leu, Val, Ile, Gly, Abu;

AXX_6 is MeAla, Sar, MeLeu; and

AXX_7 is Gly, Ala;

for use in a method for treating and/or preventing cell death associated disorders and diseases, related to the MPTP opening, and wherein said cycloundecadepsipeptide compound of Formula (I) comply with the two following criteria:

(1) MPTP inhibitory activity at least equal to half of the CsA activity, defined as:

$$\frac{Compound\_of\_formula(I)\_CRC\_IC_2}{CsA\_CRC\_IC_2} \leq 2$$

(2) at least three-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 3$$

Preferably, said cycloundecadepsipeptide compound of Formula (I) is at least 5 fold less cytotoxic than CsA, and even more preferably at least ten-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 10$$

Calcium retention capacity (CRC) is a sensitive measure of the propensity of mitochondria to open the Permeability Transition Pore (MPTP) after Ca^{2+} uptake. The IC_2 (nM) is the concentration of compound necessary to double the CRC, relative to the CRC observed in the absence of any compound (CRC_0).

According to IUPAC admitted definition, cyclodepsipeptides are natural or synthetic compounds having sequences of amino and hydroxyl carboxylic acid residues (usually α-amino and α-hydroxy acids) and the residues are connected in a ring (see IUPAC Compendium of Chemical Terminology, 2nd Edition (1997). Such cyclodepsipeptides are depicted as heterodetic peptides in which at least one amide bond has been replaced with an ester bond.

The compounds of the present invention comprise eleven residues, ten being α-amino acids and one being α-hydroxy acid. This α-hydroxy acid is (2R)-2-hydroxy-3-methyl-butanoic acid, also known as D-α-hydroxyisovaleric acid and abbreviated as H-D-Hiv-OH. In Formula (I), this hydroxyl acid is in position 8. It forms on the carboxylic acid end an amide bond with the amino group of the

α-amino acid in position 9, namely N-methyl-leucine, and, on the hydroxyl end an ester bond with the carboxylic acid group of the α-amino acid in position 7, namely alanine or glycine.

The α-amino acids of Formula (I) are mentioned using the three letter code abbreviation usually used to name amino acids and their configuration is L-configuration, unless otherwise specified. The residue numbering starts from AXX_1 representing N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine or MeBmt and its structural derivatives as defined above. When an alkyl group such as a methyl group Me or an ethyl group Et appears before the abbreviation of an amino acid, this means that such an alkyl group is fixed on the amino group of said amino acid residue.

An advantage of the compounds according to the present invention may lie in the impact of an ester bond within macro cyclic backbone in comparison to the regular cyclic amide backbone of the corresponding cycloundecapeptide compounds described in the prior art. Without being bound to theory, it is believed that the replacement of an amide by an ester bond between amino-acid AXX_7 in position 7 and D-HIV in position 8 results in a strong effect upon the conformational and physico-chemical properties such as increased conformational flexibility and lipophilicity as well as the absence of a hydrogen donor bond.

It is believed that these structural features transform to pronounced differences in the physico-chemical, pharmaco-kinetic and biological properties of the compounds of invention compared to the class of CsA derived analogues.
A possible rational for the observed higher tolerance to amino acid substitutions in systematic SAR studies may reside in an increase of the conformational space with respect to the bioactive conformation.

In contrast to cycloundecapeptide analogues of the prior art, single or multiple replacements at positions in fragment between amino-acid in position 2 and amino-acid in position 7 of the natural cycloundecadepsipeptide, as obtained either from US 5,116,816, Example 2, or from WO 02/092033, Example 4, Step 4-1, result in the retention of high binding capacities to Cyclophilin as evidenced by

the list of compounds meeting the criteria of the present invention. Most notably, an increase in the binding affinities to Cyp A of factors up to 2-4 for the compounds of the invention compared to compound according to WO 2006/038088 i.e. $[D\text{-}MeAla]^3$-$[EtVal]^4$-CsA (Alisporivir) has been observed.

Apart from the improved active profile, the new class of compounds also offers an improved preparation process, especially at an industrial scale.

The invention also encompasses chemical modifications of the compounds of formula I to prolong their circulating lifetimes. Examples of suitable poly(ethylene glycol) derivatives that possess this property are described in e.g. US 2005171328 (NEKTAR THERAPEUTICS AL CORP) or US 6,713,454 (NOBEX CORP).

More preferably, the compounds of the present invention are defined by Formula (I) in which

AXX_1 is MeBmt;

AXX_2 is Abu, Val;

AXX_3 is D-MeAla;

AXX_4 is MeIle, MeVal, EtVal;

AXX_5 is Leu, Val, Ile, Gly, Abu;

AXX_6 is MeAla, Sar, MeLeu; and

AXX_7 is Gly, Ala.

According to one of the best mode of the invention, compounds of Formula (I) present the following formulae:

Compound	Cycloundecadepsipeptide Sequence
001	Cyclo-(MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal)
009	Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal)
018	Cyclo-(MeBmt-Val-D-MeAla-MeVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal)
020	Cyclo-(MeBmt-Thr-D-MeAla-MeVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-

	Leu-MeVal)
036	Cyclo-(MeBmt-Abu-D-MeAla-MeLys(Boc)-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal)
044	Cyclo-(MeBmt-Val-D-MeAla-MeVal-Val-MeAla-Ala-D-Hiv-MeLeu-Leu-MeVal)
045	Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Val-MeAla-Ala-D-Hiv-MeLeu-Leu-MeVal)
049	Cyclo-(MeBmt-Val-D-MeAla-EtVal-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)
050	Cyclo-(MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)
051	Cyclo-(MeBmt-Val-D-MeAla-EtVal-Leu-MeAla-Ala-D-Hiv-MeLeu-Leu-MeVal)
052	Cyclo-(MeBmt-Abu-D-MeAla-EtVal-Leu-MeAla-Ala-D-Hiv-MeLeu-Leu-MeVal)
055	Cyclo-(MeBmt-Val-D-MeAla-MeVal-Ile-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)
056	Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Ile-MeAla-Gly-D-Hiv-MeLeu-Leu-MeVal)
057	Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)
058	Cyclo-(MeBmt-Abu-D-MeAla-MeThr(*t*Bu)-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal)
062	Cyclo-(MeBmt-Val-D-MeAla-MeVal-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)
064	Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Ile-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)
065	Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Val-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)
067	Cyclo-(MeBmt-Val-D-MeAla-MeVal-Gly-Sar-Gly-D-Hiv-MeLeu-Leu-MeVal)
070	Cyclo-(MeBmt-Val-D-MeAla-MeVal-Val-Sar-Gly-D-Hiv-MeLeu-Leu-MeVal)

071	Cyclo-(MeBmt-Val-D-MeAla-MeVal-Abu-Sar-Gly-D-Hiv-MeLeu-Leu-MeVal)
073	Cyclo-(MeBmt-Abu-D-MeAla-MeIle-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)

More preferably, compounds of Formula (I) are selected among:

Compound	Cycloundecadepsipeptide Sequence
009	Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal)
057	Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)

The above listed compounds correspond to an overall optimization of the most important criteria of the present invention, namely the reduction of cytotoxicity and the enhancement of anti-cell death activity. Notably, these compounds demonstrate a much lower cytotoxicity and enhanced anti-cell death activity, as compared to cyclosporine A (CsA).

The compounds of the present invention have reduced cytotoxicity, which allows (1) possible use of higher doses to achieve a maximal effect; (2) increased therapeutic window and (3) no lack of activity at high doses.

MPTP opening is a key event in the biochemical signaling that underlies mitochondria mediated cell death in various tissues, disorders and diseases. Preferably the cell death associated disorders and diseases, wherein the MPTP opening is involved, are selected from the group comprising ischaemia-reperfusion injuries, metabolic and/or oxidative stress disorders, diseases involving loss of cellular calcium homeostasis, age related cellular degeneration, diseases involving toxic and/or infectious pathogens.

More preferably, the cell death associated disorders and diseases, related to the MPTP opening, are selected from the group comprising ischaemia and ischaemia-

reperfusion injuries, muscular dysthrophies, neurodegeneration disorders and diseases, West Nile viral encephalitis, Japanese encephalitis, Chronic hepatitis and cirrhosis induced by Hepatitis C virus, Hepatitis B, delta agent, acute and chronic drug- or toxin-induced hepatotoxicity, alcoholic liver disease, cholestasis, Non Alcoholic Fatty Liver Disease, gastro-intestinal ulcerations caused by NSAIDs, transplant rejection, peripheral vascular insufficiency, renal insufficiency, obesity, diabetic micro-angiopathy, diabetic nephropathy, Maturity onset diabetes of the young type 4, acute and chronic pancreatitis, septicaemia, multi organ dysfunction syndrome, crush syndrome, congestive heart failure, atherosclerosis, hypertensive heart disease, cancer, bipolar disorder.

Even more preferably,

Ischaemia and ischaemia-reperfusion injuries are selected from the group comprising acute cerebral ischemia, acute stroke, acute myocardial infarction cardiac arrest, myocardial stunning, post-reperfusion arrhythmias, organ failure following therapeutic procedures such as thrombolytic therapy, coronary angioplasty, aortic cross-clamping or coronary bypass surgery, hypovolemic shock, storage/reperfusion injury of transplant organs, multi organ dysfunction syndrome.

Muscular dysthrophies are selected from the group comprising Ulrich congenital muscular dysthrophy, Bethlem myopathy, myosclerosis, limb girdle muscular dysthropy, Duchenne muscular dysthrophy, Becker muscular dysthrophy, Emery-Dreifuss syndrome,

Neurodegeneration disorders and diseases are selected from the group comprising traumatic brain injury, spinal cord injury, peripheral nerve injury, epilepsy-induced brain injury, amyothrophic lateral sclerosis (ALS), Alzheimer disease, Parkinson disease , multiple sclerosis, Huntington disease, axonal degeneration-induced neuropathic pain, diabetic neuropathy chemotherapy-induced neuropathic pain, or herpes-induced neuropathy.

Most preferably, the cell death associated disorders and diseases, related to the MPTP opening, are selected among acute and chronic pancreatitis and muscular dystrophy

Pancreatitis is an inflammation of the pancreas that can occur in two very different forms. Acute pancreatitis is sudden while chronic pancreatitis is characterized by recurring or persistent abdominal pain with or without steatorrhea or diabetes mellitus.

Acute pancreatitis is a severe and frequently lethal disorder most commonly caused by gallstone disease and alcohol abuse, whereas chronic pancreatitis is a long-standing inflammation of the pancreas that alters its normal structure and functions. Acute pancreatitis is characterized by acinar cell necrosis which contributes to pancreatic and extra-pancreatic autodigestion as well as systemic inflammatory response and multiple organ failure. A key event in acinar cell necrosis is the formation of the MPTP within the inner mitochondrial membrane leading to permeabilization of the mitochondrial membrane. This causes rapid depolarization of the mitochondrial membrane potential and uncouples ATP synthesis from oxidative metabolism leading to ATP depletion and necrosis (Mukherjee, R., *et al.*, 2008). Thus by inhibiting the formation of the MPTP, the compounds have the ability to prevent acinar cell necrosis and attenuate the pancreatic injury.

Muscular dystrophies comprise a diverse group of genetic disorders that lead to muscle wasting and, in many instances, premature death. It was demonstrated (Millay et al., 2008) that mitochondrial-dependent necrosis represents a prominent disease mechanism in muscular dystrophy. Indeed one major mechanism leading to cellular necrosis is mitochondrial calcium overload, which secondarily enhances reactive oxygen species (ROS) generation and further promotes MPT (Mitochondrial Permeability Transition). Therefore the inhibition of MPTP opening provides a new treatment strategy for muscular dystrophies.

The compounds of the present invention may be obtained by applying classical peptide (solution or solid-phase peptide synthesis; in Houben – Weyl, Methods of

Organic Chemistry, Vol. E 22d, Ed.-in-Chief: M. Goodman, Thieme Verlag, Stuttgart, 2003) and organic chemistry or biotechnology, for instance by applying the chemical tools as described by Wenger in Helv. Chim. Acta, 67, 502-25, 1984 or in Helv. Chim. Acta, 66, 2672-702 (1983) and by employing HATU as coupling reagent described in Rich, D.H. et al.. Comparative studies of the coupling of N-methylated, sterically hindered amino acids during SPPS, Tetr. Letters. 35, 5981-5984 (1994).

For instance, one of possible general scheme consists to prepare two fragments, namely Fragment A and Fragment B, containing the appropriate residues, with, when necessary the appropriate protective and activating groups, and, in the last steps of the preparation, to link them together to obtain an undecapeptide which is then cyclized to the cycloundecadepsipeptide.

For instance, Fragment (Ax) may be as follows:
H-D-Hiv-MeLeu-Leu-MeVal-AXX_1-OH (Ax)
and Fragment (Bx) as follows :
H-AXX_2-AXX_3-AXX_4-AXX_5-AXX_6-AXX_7-OR, (R being an alkyl group) (Bx),
then Ax and Bx are coupled to the undecadepsipeptide Ax-Bx, H-D-Hiv-MeLeu-Leu-MeVal-AXX_1-AXX_2-AXX_3-AXX_4-AXX_5-AXX_6-AXX_7-OR (R= alkyl,H)
and the final stage is the macrolactonisation.

Fragment A, which contains the α-hydroxy acid residue D-Hiv, may be obtained by degradation of a natural cycloundecadepsipeptide (namely Cyclo-(MeBmt-Thr-Sar-MeLeu-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal), the preparation of said cycloundecadepsipeptide being described either in US 5,116,816, Example 2, or in WO 02/092033, Example 4, Step 4-1.

An advantage of the compounds according to the invention versus the prior art lies not only in its active profile (Example – Experimental Results), but also in its improved preparation process especially at an industrial scale.

1. Starting from the natural compounds (CsA and Cyclo-(MeBmt-Thr-Sar-MeLeu-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal), the overall yields for the synthesis of

cycloundecadepsipeptide analogues are > 50% compared to 20% for ALISPORIVIR ([D-MeAla]^3-[EtVal]^4-CsA as described in WO 2006/038088, DEBIOPHARM SA).

2. The synthesis of the expensive dipeptide derivative Boc-D-MeAla-EtVal-OH (including 4 chemical steps) is not needed. In addition, some of the compounds contain a C-terminal Glycine, which facilitates the last step of the synthesis (macrolactonisation, no epimerisation).

3. The costs for reagents and starting compounds are considerably lower in case of the compounds according to the invention. Notably, the “reagent of Meerwein” used for the ring opening reaction of CsA is not needed.

4. Fragment B (hexapeptide AXX_2-AXX_7) can be efficiently obtained by standard solid-phase peptide synthesis using commercially starting compounds.

5. In optimizing the preparation of Fragment A from the natural starting compound as well as the condensation (fragment A with fragment B), macrocyclization and final purification steps, the overall yield for obtaining the pharmacologically interesting compounds can amount up to 80%.

6. Overall, the indicated aspects result in a good efficacy and consequently in cost of goods, in particular with respect to the synthesis of the compounds of invention at industrial scale.

The present invention further provides a pharmaceutical composition for treating and/or preventing cell death associated disorders or diseases, related to the MPTP opening, comprising a compound of the invention, together with one or more pharmaceutically acceptable diluents or carriers.

The compounds of the present invention may be administrated for instance parenterally or orally to a patient in need, for instance incorporated in a pre-concentrated microemultion.

The compounds of the present invention, their pharmaceutically acceptable salts and pro-drugs thereof, where applicable, may be administered in the form of a pharmaceutical composition in which they are in association with a pharmaceutically acceptable adjuvant, diluent or carrier, in order to prevent or treat cell death associated disorders or diseases, wherein the MPTP opening is involved.

The present invention also provides a pharmaceutical composition comprising a compound of the present invention, or a pharmaceutically acceptable salt thereof, as hereinbefore defined, in association with a pharmaceutically acceptable adjuvant, diluent or carrier. As to the appropriate excipients, diluents and adjuvants, reference may be made to the standard literature describing these, e.g. to chapter 25.2 of Vol. 5 of "Comprehensive Medicinal Chemistry", Pergamon Press 1990, and to "Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete", by H.P. Fiedler, Editio Cantor, 2002.

The compounds of the present invention may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, *Osol, A. Ed.* (1980). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi permeable matrices of solid hydrophobic polymers containing the compounds of the present invention, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and [gamma] ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as those used to prepare the medicament LUPRON DEPOT(TM) (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The daily dose of the present invention will necessarily be varied depending upon the host treated, the particular route of administration, and the severity and kind of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

The pharmaceutical compositions of the invention may be formulated as creams, gels, solutions, ointments, suspensions or plasters *etc*. when intended for topical administration; for administration by inhalation, e.g. as aerosols or dry powders; for oral administration, e.g. in the form of tablets, capsules, gels, syrups, suspensions, solutions, powders or granules; for rectal or vaginal administration *e.g*. as suppositories; or for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular, intrathecal or infusion) as a sterile solution, suspension or emulsion.

The active compound of the invention may be administered by any conventional route. It may be administered parentally, e.g., in the form of injectable solutions or suspensions, or in the form of injectable deposit formulations. Preferably, it will be administered orally in the form of solutions or suspensions for drinking, tablets or capsules. The pharmaceutical compositions of the invention typically comprise a cycloundecadepsipeptide compound of the invention and one or more pharmaceutically acceptable carrier substances. Typically, these compositions are concentrated and need to be combined with an appropriate diluent, e.g., water, prior to administration. Pharmaceutical compositions for parenteral administration typically also include one or more excipients. Optional excipients include an isotonic agent, a buffer or other pH- controlling agent, and a preservative. These excipients may be added for maintenance of the composition and for the attainment of preferred ranges of pH (about 6.5-7.5) and osmolarity (about 300 mosm/L). Additional examples of formulations for oral administration can be found in U.S. Pat. Nos. 5,525,590 and 5,639,724, and U.S. Pat. Appl. 2003/0104992. By the oral route, the indicated dosage of a cycloundecadepsipeptide compound of the invention for daily to trice weekly administration may be from about 1 mg/kg to about 100 mg/kg, preferably from about 1 mg/kg to about 20 mg/kg. By the intravenous route, the indicated corresponding dosage may be from about 1 mg/kg

to about 50 mg/kg, preferably from about 1 mg/kg to about 25 mg/kg. An effective amount of a cycloundecadepsipeptide compound of the invention is understood to be an amount that when administered in the course of a therapeutic regimen to a patient in need of treatment of MPTP opening mediated disorder results in an objective clinical response.

Numerous factors will be taken into consideration by a clinician when determining trial doses for testing efficacy of a pharmaceutical composition comprising a compound of the present invention against MPTP opening mediated disorders. Primary among these are the toxicity and half-life of the chosen cycloundecadepsipeptide compound of the invention. Additional factors include the weight of the patient, the age of the patient, and the general condition of the patient. A course of treatment will require repeated administration of a pharmaceutical composition of the invention. Typically, an adequate drug dose will be administered in one single administration (i.e. a unique dose) or repeatedly for example 3-7 times per week, and the duration of treatment may be for example from about 4 weeks to 6 months, preferably from about 4 weeks to about 12 months.

A pharmaceutical composition of the present invention may comprise one or more other active ingredients which present MPTP inhibitory activity in addition to the compounds of the present invention. Compounds of the invention and such other active ingredients can be administered together as part of the same pharmaceutical composition or can be administered separately as part of an appropriate dose regimen designed to obtain the benefits of the combination therapy. The appropriate dose regimen, the amount of each dose administered, and specific intervals between doses of each active agent will depend upon the specific combination of active agents employed, the condition of the patient being treated, and other factors discussed in the previous section. Such additional active ingredients will generally be administered in amounts less than or equal to those for which they are effective as single therapeutic agents. The FDA approved dosages for such active agents that have received FDA approval for administration to humans are publicly available.

The compounds of the invention may be administered as the sole ingredient or together with other drugs.

Also encompassed is a method for preventing or treating cell death associated disorders or diseases, related to the MPTP opening, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of the pharmaceutical composition according to claim 4 or the cycloundecadepsipeptide compound of Formula (I)

Cyclo-(AXX$_1$-AXX$_2$-AXX$_3$-AXX$_4$-AXX$_5$-AXX$_6$-AXX$_7$-D-Hiv-MeLeu-Leu-MeVal) (I)

1 2 3 4 5 6 7 8 9 10 11

in which:

AXX$_1$ is MeBmt, 4-fluoro-MeBmt, dihydro-MeBmt, 8-hydroxy-MeBmt; O-acetyl-MeBmt;

AXX$_2$ is Abu, Val, Thr, Thr(OMe), Thr(OAc), Thr(OCOCH$_2$CH$_2$CH$_2$OH), Nva, 5-hydroxy-Nva;

AXX$_3$ is D-MeAla, D-3-fluoro-MeAla, D-MeSer, D-MeSer(OAc), D-MeSer(OCH$_2$CH$_2$OH), D-MeSer(OCH$_2$CH$_2$NEt$_2$), D-MeAsp(OMe);

AXX$_4$ is MeIle, MeMet, MeVal, MeThr, MeThr(OAc), MeAla, EtVal, EtIle, EtPhe, EtTyr, EtThr(OAc), MeThr(OAc), MeTyr, MeTyr(OAc), MeTyr(OMe), MePhe, MeMet(Ox) wherein the sulphur atom of methionine is sulphoxyde or sulphone;

AXX$_5$ is Leu, Val, Ile, Gly, Abu;

AXX$_6$ is MeAla, Sar, MeLeu; and

AXX$_7$ is Gly, Ala;

and wherein said cycloundecadepsipeptide compound of Formula (I) comply with the two following criteria:

(1) MPTP inhibitory activity at least equal to half of the CsA activity, defined as:

$$\frac{Compound\_of\_formula(I)\_CRC\_IC_2}{CsA\_CRC\_IC_2} \leq 2$$

(2) at least three-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 3$$

Preferably, said cycloundecadepsipeptide compound of Formula (I) is at least 5 fold less cytotoxic than CsA, and even more preferably at least ten-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 10$$

Preferably the cell death associated disorders and diseases, wherein the MPTP opening is involved, are selected from the group comprising ischaemia-reperfusion injuries, metabolic and/or oxidative stress disorders, diseases involving loss of cellular calcium homeostasis, age related cellular degeneration, diseases involving toxic and/or infectious pathogens.

More preferably, the cell death associated disorders and diseases, related to the MPTP opening, are selected from the group comprising ischaemia and ischaemia-reperfusion injuries, muscular dysthrophies, neurodegeneration disorders and diseases, West Nile viral encephalitis, Japanese encephalitis, Chronic hepatitis and cirrhosis induced by Hepatitis C virus, Hepatitis B, delta agent, acute and chronic drug- or toxin-induced hepatotoxicity, alcoholic liver disease, cholestasis, Non Alcoholic Fatty Liver Disease, gastro-intestinal ulcerations caused by NSAIDs, transplant rejection, peripheral vascular insufficiency, renal insufficiency, obesity, diabetic micro-angiopathy, diabetic nephropathy, Maturity onset diabetes of the young type 4, acute and chronic pancreatitis, septicaemia, multi organ dysfunction syndrome, crush syndrome, congestive heart failure, atherosclerosis, hypertensive heart disease, cancer, bipolar disorder.

Even more preferably,

Ischaemia and ischaemia-reperfusion injuries are selected from the group comprising acute cerebral ischemia, acute stroke, acute myocardial infarction cardiac arrest, myocardial stunning, post-reperfusion arrhythmias, organ failure

following therapeutic procedures such as thrombolytic therapy, coronary angioplasty, aortic cross-clamping or coronary bypass surgery, hypovolemic shock, storage/reperfusion injury of transplant organs, multi organ dysfunction syndrome.

Muscular dysthrophies are selected from the group comprising Ulrich congenital muscular dysthrophy, Bethlem myopathy, myosclerosis, limb girdle muscular dysthropy, Duchenne muscular dysthrophy, Becker muscular dysthrophy, Emery-Dreifuss syndrome,

Neurodegeneration disorders and diseases are selected from the group comprising traumatic brain injury, spinal cord injury, peripheral nerve injury, epilepsy-induced brain injury, amyothrophic lateral sclerosis (ALS), Alzheimer disease, Parkinson disease , multiple sclerosis, Huntington disease, axonal degeneration-induced neuropathic pain, diabetic neuropathy chemotherapy-induced neuropathic pain, or herpes-induced neuropathy.

Most preferably, the cell death associated disorders and diseases, related to the MPTP opening, are selected among acute and chronic pancreatitis and muscular dystrophy

Another object of the invention is a method, comprising co-administration concomitantly or in sequence of a therapeutically effective amount of the compound according to the invention or the pharmaceutical composition of the invention and a co-agent selected from an agent having blocking properties on MPTP opening.

The administration of a pharmaceutical combination of the invention results in a beneficial effect, e. g. a synergistic therapeutic effect, compared to a monotherapy applying only one of its pharmaceutical active ingredients. For instance, a synergistic combination is for example a combination of the cycloundecadepsipeptide compound of the invention with an interferon, optionally conjugated to a polymer.

All patents, patent applications and publications cited herein shall be considered to have been incorporated by reference in their entireties.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications without departing from the spirit or essential characteristics thereof. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features. The present disclosure is therefore to be considered as in all aspects illustrated and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

Various references are cited throughout this Specification, each of which is incorporated herein by reference in its entirety.

The foregoing description will be more fully understood with reference to the following Examples. Such Examples, are, however, exemplary of methods of practicing the present invention and are not intended to limit the scope of the invention.

Examples

Example 1:

Preparation of cycloundecadepsipeptide (compound 001):

cyclo-(MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (001)

1. Preparation of Fragment (Aa), starting from natural cycloundecadepsipeptide Cyclo-(MeBmt-Thr-Sar-MeLeu-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal)

(see US 5,116,816, Example 2, or in WO 02/092033), Example 4, Step 4-1):

H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-OH (Aa)

1.1. Preparation of Cyclo-(MeBmt-Thr(O-(N-Imidazolyl)carbonyl)-Sar-MeLeu-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (1A).

A solution of Cyclo-(MeBmt-Thr-Sar-MeLeu-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (US 5,116,816, Example 2, or in WO 02/092033, Example 4, Step 4-1) (3.00 g, 2.40 mmol, 1.0 equiv.) and 1,1′-carbonyldiimidazole (1.17 g, 7.21 mmol, 3.0 equiv.) in 15 mL of anhydrous CH_2Cl_2 was stirred at room temperature for 2.5 h. Progress of the reaction was monitored by analytical UPLC. 81.1% conversion was obtained. Additional amount of 1,1′-carbonyldiimidazole (0.39 g, 2.40 mmol, 1.0 equiv.) was added to the reaction mixture. After additional stirring for 16 h, a 98.8% conversion was obtained. The solution was evaporated under reduced pressure. The residue was dissolved in AcOEt (45 mL) and washed successively with 10% citric acid (45 mL) and brine (45 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a mixture of two compounds (**1A**) and (**2A**) as a white powder
UPLC-ESI-MS (m/z): (**1A**): 1342.75 $[M + H]^+$ ($[C_{68}H_{117}N_{12}O_{15}]^+$; calc. 1342.73), (**2A**): 1274.77 $[M + H]^+$ ($[C_{65}H_{113}N_{10}O_{15}]^+$; calc. 1274.65)

1.2. Preparation of Cyclo-(MeBmt-Thr(O,N-carbonyl)-Sar-MeLeu-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (2A).

A solution of compound (**1A**) as obtained in the preceding reaction (3.41 g, 2.40 mmol) in dry DMSO (15 mL) was stirred and heated at 100 °C for 2 h under argon atmosphere. Progress of the reaction was monitored by analytical UPLC. Full conversion was obtained. Subsequently, the solution was dissolved in AcOEt (45 mL) and washed successively with HCl 1M (30 mL) and 23.2% aq. NaCl solution (15 mL) then 11.6% aq. NaCl solution (45 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give the cycloundecadepsipeptide **Cyclo-(MeBmt-Thr(O,N-carbonyl)-Sar-MeLeu-Leu-**

MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (**2A**) as white powder UPLC-ESI-MS (m/z): 1274.65 $[M + H]^+$ ($[C_{65}H_{113}N_{10}O_{15}]^+$; calc. 1274.65.

1.3. Preparation of H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-OMe (fragment (Aa) methyl ester (3A).

To a solution of the cyclodepsipeptide as obtained in the preceding reaction (1.00 g, 0.78 mmol) in 30 mL of MeOH (dry), cooled at 0 °C, was added KOMe (0.24 g, 2.35 mmol) in one portion, under argon. After 5 h at 0 °C then 1 h at room temperature, a control of the reaction advancement by UPLC indicates 98.6% conversion. The solution was cooled at 0 °C then neutralized with an aq. solution of 10% citric acid. Methanol was evaporated. The aq. mixture was poured into 100 mL of a mixture of AcOEt/NaCl 23.2% (1:1 $^{V}/_{V}$). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Crude mixture was separated by column chromatography; to give the pentapeptide **-D-Hiv-MeLeu-Leu-MeVal-MeBmt-OMe** (**3A**

UPLC-ESI-MS (m/z): 669.16 $[M + H]^+$ ($[C_{35}H_{65}N_4O_8]^+$; calc. 669.48).

1.4. Preparation of Fragment (Aa) H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-OH (**4**).

A solution of the pentapeptide as obtained in the preceding reaction (**3A**) (1.15 g, 1.72 mmol, 1.0 equiv.) in THF (8.6 mL) and water (1.1 mL) was cooled to 0 °C in an ice-water bath, then 2 M LiOH (1.72 mL, 3.44 mmol, 2.0 equiv.) was added within 20 seconds. The cooling bath was then removed and the mixture (pH = 12-13) was stirred at room temperature for about 3 h. A control of the reaction advancement by UPLC indicates a full conversion. Subsequently, the solution was cooled to 0 °C and neutralized with 10% citric acid. Tetrahydrofurane was evaporated under reduced pressure. The residue was dissolved in AcOEt (50 mL) and washed with 10% citric acid (3 mL) and 23.2% aq. NaCl solution (50 mL) (pH 3). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure to give **Fragment (Aa) H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-OH** (**4**) as a white powder

UPLC-ESI-MS (m/z) 655.21 $[M + H]^+$ ($[C_{34}H_{63}N_4O_8]^+$; calc. 655.46).

2. Preparation of Fragment (Bb) :

H-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe (Bb)

2.1. Preparation of Boc-MeLeu-Ala-OMe

To a solution of commercially available H-Ala-OMe·HCl (2.00 g, 14.32 mmol, 1.0 equiv.) in dry DCM (130 mL), cooled at 0 °C, was added under argon DIPEA (14.68 mL, 86.0 mmol, 6.0 equiv.). Subsequently, after 15 min, HATU (6.52 g, 17.18 mmol, 1.2 equiv.) and Boc-MeLeu-OH (3.512 g, 14.32 mmol, 1.0 equiv.) were added in one portion. After 15 min at 0 °C then 48 h at room temperature (RT = 2 days 15 min), a control of the reaction advancement by TLC indicates the completion. The reaction was quenched by addition of 10% $NaHCO_3$ (20 mL) and stirred 15 min. Reaction mixture was diluted with DCM (200 mL) and washed with 10% citric acid (1 × 40 mL), H_2O (1 × 40 mL) and brine (1 × 40 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Crude mixture was separated by CC to give the ester Boc-MeLeu-Ala-OMe as a pale yellowish oil. The sample (30 mg) was repurified by semi-preparative RP-HPLC and the peptide was lyophilized to give the ester Boc-MeLeu-Ala-OMe as a white powder.

2.2. Preparation of H-MeLeu-Ala-OMe

A solution of dipeptide as obtained in the preceding reaction (1.70 g, 5.14 mmol, 1.0 equiv.) in TFA/DCM (10 mL, 2:3 $^{V}/_{V}$) was kept at 0 °C for 1.5 h, and the solvents removed under reduced pressure. The crude product was dried under high vacuum (20 min). Subsequently, DCM (10 mL) was added and the mixture was triturated at 0 °C with DIPEA to pH 7-8 to neutralize an excess of TFA. Subsequently, reaction mixture was diluted with DCM (60 mL) and washed with H_2O (1 × 10 mL) and brine (1 × 10 mL). The organic phase was filtered, dried (Na_2SO_4), evaporated and dried under high vacuum. The crude amine salt of H-MeLeu-Ala-OMe was used for the next coupling step without further purification.

2.3. Preparation of Boc-Leu-MeLeu-Ala-OMe

To a solution of dipeptide as obtained in the preceding reaction (7) (2.17 g, 9.42 mmol, 1.0 equiv.) in dry DCM (108 mL), cooled at 0 °C, was added under argon DIPEA (6.45 mL, 37.68 mmol, 4.0 equiv.). Subsequently, after 10 min, HATU (4.65 g, 12.25 mmol, 1.3 equiv.) and Boc-Leu-OH (2.39 g, 10.36 mmol, 1.1 equiv.) were added in one portion. After 15 min at 0 °C then 15 h at room temperature (15 h 15 min), a control of the reaction advancement by TLC indicates the completion. The reaction was quenched by addition of 10% $NaHCO_3$ (10 mL) and stirred 15 min. Reaction mixture was diluted with DCM (50 mL) and washed with 10% citric acid (1 × 20 mL), H_2O (1 × 20 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Crude mixture was separated by CC to give the tripeptide Boc-Leu-MeLeu-Ala-OMe as a yellowish oil. The sample was re-purified by semi-preparative RP-HPLC and the peptide lyophilized to give the tripeptide Boc-Leu-MeLeu-Ala-OMe as a white powder.

2.4. Preparation of H-Leu-MeLeu-Ala-OMe

A solution of the tripeptide as obtained in the preceding reaction (2.00 g, 4.50 mmol, 1.0 equiv.) in TFA/DCM (10 mL, 2:3 $^{V}/_{V}$) was kept at 0 °C for 2 h, and the solvents removed under reduced pressure. The product was dried under high vacuum (20 min) to give the crude material. Subsequently, DCM (20 mL) was added and the mixture was triturated at 0 °C with DIPEA to pH 7-8 (Lackmus-paper) to neutralize an excess of TFA. After evaporation and drying the crude amine H-Leu-MeLeu-Ala-OMe was used to the next coupling step without further purification. The sample (25 mg) was repurified by semi-preparative RP-HPLC and the product was lyophilized to give the tripeptide H-Leu-MeLeu-Ala-OMe as a solid

2.5. Preparation of Boc-D-MeAla-EtVal-OH

Boc-D-MeAla-EtVal-OH was obtained starting from Boc-D-MeAla-Val-OMe using BuLi and Triethyloxoniumfluoroborate, followed by hydrolysis of the methylester

according to literature [Jean François. Guichou, PhD thesis entitled "De nouveaux analogues de Cyclosporine A comme agent anti-VIH-1", Faculté des Sciences, Université de Lausanne, 2001, p.121-122].

2.6. Preparation of Boc-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe

To a solution of the tripeptide H-Leu-MeLeu-Ala-OMe (0.096 g, 0.291 mmol, 1.0 equiv.) in dry DCM (8 mL), cooled at −8 °C, was added under argon DIPEA (0.15 mL, 0.87 mmol, 3.0 equiv.). Subsequently, after 10 min, HATU (0.13 g, 0.35 mmol, 1.2 equiv.) and the dipeptide Boc-D-MeAla-EtVal-OH (0.1 g, 0.29 mmol, 1.0 equiv.) were added in one portion. After 10 min at −8 °C then 40 min at room temperature, a control of the reaction advancement by HPLC indicates the completion. The reaction was quenched by addition of 10% $NaHCO_3$ (1 mL) and stirred 15 min. Reaction mixture was diluted with DCM (20 mL) and washed with 10% citric acid (1 × 5 mL), H_2O (1 × 5 mL) and brine (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude mixture was separated by CC to give the pentapeptide Boc-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as an oil. The sample was repurified by semi-preparative RP-HPLC and the product was lyophilized to give the pentapeptide Boc-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as a white powder.

2.7. Preparation of H-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe

A solution of the pentapeptide as obtained in the preceding reaction (0.45 g, 0.69 mmol, 1.0 equiv.) in TFA/DCM (4.0 mL, 2:3 $^{V}/_{V}$) was kept at 0 °C for 1.5 h, and the solvents removed under reduced pressure. The residue was dried under high vacuum (0.5 h). Subsequently, DCM (10 mL) was added and the mixture was triturated at 0 °C with DIPEA to pH 7-8 (Lackmus-paper) to neutralize an excess of TFA. After evaporation and drying the crude amine salt of the pentapeptide H-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe was used to the next coupling step without further purification. A sample (30 mg) was purified by semi-preparative RP-HPLC and the peptide was lyophilized to give the free amine of the pentapeptide H-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as a white powder.

2.8. Preparation of Boc-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe

To a solution of the crude pentapeptide as obtained in the preceding reaction (0.2 g, 0.36 mmol, 1.0 equiv.) in dry DCM (10 mL), cooled at 0 °C, was added under argon DIPEA (0.18 mL, 1.08 mmol, 3.0 equiv.). Subsequently, after 5 min, HATU (0.19 g, 0.5 mmol, 1.4 equiv.) and Boc-Abu-OH (0.088 g, 0.42 mmol, 1.1 equiv.) were added in one portion. After 15 min at 0 °C then 1.15 h at room temperature (RT = 1.5 h), a control of the reaction advancement by TLC indicates the completion. The reaction was quenched by addition of 10% $NaHCO_3$ (2 mL) and stirred 15 min. Reaction mixture was diluted with DCM (50 mL) and washed with 10% citric acid (1 × 10 mL), H_2O (1 × 10 mL) and brine (1 × 10 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Crude mixture was separated by CC to give the hexapeptide Boc-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as a yellowish oil. A sample (30 mg) was repurified by semi-preparative RP-HPLC and the product lyophilized to give the hexapeptide Boc-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as a white powder.

2.9. Preparation of H-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe Fragment (Bb)

A solution of the hexapeptide as obtained in the preceding reaction (0.187 g, 0.25 mmol, 1.0 equiv.) in TFA/DCM (3.0 mL, 2:3 $^{V}/_{V}$) was kept at 0 °C for 1.5 h, and the solvents removed under reduced pressure. The crude product was dried under high vacuum (0.5 h). Subsequently, DCM (3 mL) was added and the mixture was triturated at 0 °C with DIPEA to pH 7-8 (Lackmus-paper) to remove TFA. After evaporation and drying the crude amine H-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe was used to the next coupling step without further purification. A sample of crude product (23 mg) was re-purified by semi-preparative RP-HPLC and the peptide was lyophilized to give Fragment (Ba) H-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as a white powder.
UPLC-ESI-MS (m/z) 641.259 $[M + H]^+$ ($[C_{32}H_{61}N_6O_7]^+$; calc.641.4602).

3. Coupling of fragment (Aa) and fragment (Bb)

3.1. Preparation of H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe

To a solution of the hexapeptide H-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as obtained in the preceding reaction (0.16 g, 0.25 mmol, 1.0 equiv.) in dry DCM (10 mL), cooled at 0 °C, was added under argon DIPEA (0.214 mL, 1.26 mmol, 5.0 equiv.). Subsequently, after 5 min, HATU (0.12 g, 0.31 mmol, 1.25 equiv.) and the pentadepsipeptide H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-OH (4) as obtained above (0.16 g, 0.25 mmol, 1.0 equiv.) were added in one portion. After 15 min at 0 °C then 1.15 h at room temperature (RT = 1.5 h), a control of the reaction advancement by TLC indicates the completion. The reaction was quenched by addition of 10% $NaHCO_3$ (3 mL) and stirred 15 min. Reaction mixture was diluted with DCM (40 mL) and washed with 10% citric acid (1 × 8 mL), H_2O (1 × 8 mL) and brine (1 × 8 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Crude mixture was separated by CC to give the undecapeptide H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as a white foam. An analytical sample (18 mg) was repurified by semi-preparative RP-HPLC and the product was lyophilized to give the undecapeptide H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OMe as a white powder.

3.2. Preparation of H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OH

A solution of the undecapeptide methylester as obtained in the preceding reaction (0.27 g, 0.21 mmol, 1.0 equiv.) in THF (2.5 mL) was cooled to 0 °C in an ice-water bath, then 0.2 M LiOH (2.11 mL, 0.42 mmol, 2.0 equiv.) was added dropwise over 10 min. The cooling bath was then removed and the mixture (pH = 12−13; Lackmus-paper) was stirred at room temperature for 1 h 20 min (TLC control). Subsequently, the solution was cooled to 0 °C and acidified with 1.0 M HCl to pH 3−4. The solution was evaporated under reduced pressure. The residue was

dissolved in AcOEt (40 mL) and washed with 10% citric acid (1 × 8 mL), H_2O (1 × 8 mL) and brine (1 × 8 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude mixture was separated by CC to give the acid H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OH as a white powder. The sample (40 mg) was repurified by semi-preparative RP-HPLC and the product was lyophilized to give the undecapeptide H-D-Hiv-MeLeu-Leu-MeVal-MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-OH as a white powder.

4. Macrolactonisation : Preparation of cyclo-(MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (compound 001)

To a solution of DMAP (0.048 g, 0.39 mmol, 4.0 equiv.) and benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (0.102 g, 0.196 mmol, 2.0 equiv.) in DCM (12 mL) was added dropwise over 1.5 h a solution of the undecapeptide as obtained in the preceding reaction (0.124 g, 0.098 mmol, 1.0 equiv.) in DCM (4 mL). The mixture was stirred after the addition of acid was complete (RT = 24 h), then transferred to a separating funnel. The solution was washed with 1 M HCl (2 mL) and the organic layer was separated and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography, thereby giving the cycloundecadepsipeptide cyclo-(MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (Ia) as a white solid. A sample (77 mg) was purified by semi-preparative RP-HPLC and the product was lyophilized to give the cycloundecadepsipeptide cyclo-(MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (Ib) as a white powder.
UPLC-ESI-MS (m/z) 1245.787 $[M + H]^+$ ($[C_{65}H_{117}N_{10}O_{13}]^+$; calc. 1245.8802), 623.465 $[M/2 + H]^+$ (calc. 623.444).

Other cycloundecadepsipeptide compounds of Formula (I) according to the invention can be prepared according to analogous reaction pathway as described above or any other techniques known to the skilled in the art.

Example 2: **BIOLOGICAL DATA**

MPTP inhibitory activity

The MPTP activity was evaluated by measuring the Calcium Retention Capacity (CRC) of mouse liver mitochondrial preparations. CRC is a sensitive measure of the propensity of mitochondria to open the Permeability Transition Pore (MPTP) after Ca2+ uptake. CRC of mitochondria was assessed fluorimetrically using the Ca2+ indicator Calcium Green-5N. Mitochondria was incubated 1 min with ranging concentrations of the test compound and the concentration of compound necessary to double the CRC (IC_2) was determined by constructing dose-response curves.

Experimental procedure

Mitochondria were isolated from the liver of 9-10 week old male C57Bl/6j mice. After isolation the liver was kept at 4°C in a Tris-buffered (pH 7.4) sucrose-based isotonic solution and quickly minced and homogenized with a Teflon/glass potter. The suspension was spun for 6 min at 700 x *g*, and the resulting supernatant was spun for 6 min at 7,000 x *g*. The mitochondrial pellet was carefully resuspended in excess volume of sucrose buffer and spun again as above. The final mitochondrial pellet was resuspended with a minimal volume of sucrose buffer. Protein concentration was determined with the biuret method. Mitochondrial suspensions were used immediately and not beyond 4 h from isolation.

The CRC of mitochondrial preparations was assessed fluorimetrically in the presence of the Ca^{2+} indicator Calcium Green-5N at room temperature using a Fluoroskan Ascent FL fluorometer (Thermo Electron Corporation). The experiments were done in 96-well plates with 100µl of mitochondrial suspension, adding 20 µM Ca^{2+} (2µl of a 1 mM Ca^{2+} solution) every minute and monitoring Ca-green fluorescence changes at λ_{ex} 485 nm, λ_{em} 538 nm.

All compounds were dissolved in DMSO to a final concentration of 10 mM, and stored at 4°C.

Data analysis

Every treatment was represented by the mean value ± SD of (1) the CRC and (2) the ratio between CRC (as just defined) and CRC_0 (concentration of Ca^{2+} which opens the PTP in the absence of the compound).
The IC_2 (nM) was calculated as the concentration of compound necessary to double the CRC, relative to the CRC observed in the absence of any compound (CRC_0).

Cytotoxicity

Cytotoxicity was evaluated by measuring proliferation of HepG2 cells. Cells were incubated with ranging concentrations of the test compound for 72hrs. Cell proliferation is measured by the signal intensity of the incorporated nuclear dye. The half maximal inhibitory concentration (IC_{50}) was determined by constructing dose-response curves.

Experimental Procedure

Cells were grown in RPMI 1640, 10%FBS, 2 mM L-alanyl-L-Glutamine, 1mM Na Pyruvate or a special medium in a humidified atmosphere of 5% CO_2 at 37°C. Cells were seeded into 384-well plates and incubated in a humidified atmosphere of 5% CO_2 at 37°C. Compounds were added 24 hours post cell seeding. At the same time, a time zero untreated cell plate was generated.

After a 72 hour incubation period, cells were fixed and stained with nuclear dye. Automated fluorescence microscopy was carried out using a GE Healthcare IN Cell Analyzer 1000, and images were collected with a 4X objective.

Data Analysis

Twelve bit tiff images were acquired using the InCell Analyzer 1000 3.2 and analyzed with Developer Toolbox 1.6 software. The cell proliferation assay output

is referred to as the relative cell count. Relative cell count IC_{50} is the test compound concentration that produces 50% of the cell proliferation inhibitory response or 50% cytotoxicity level. IC_{50} values were calculated using nonlinear regression to fit data to a sigmoidal 4 point, 4 parameter One-Site dose response model, where: y (fit) = A + [(B – A)/(1 + ((C/x) ^ D))]. Curve-fitting and IC_{50} calculations are performed using a custom data reduction engine MathIQ based software (AIM).

Experimental Results

For selected compounds having MPTP inhibitory activity and cytotoxicity as defined in the present invention

(1) MPTP inhibitory activity at least equal to half of the CsA activity, defined as:

$$\frac{Compound\_of\_formula(I)\_CRC\_IC_2}{CsA\_CRC\_IC_2} \leq 2$$

(2) at least three-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 3$$

Compound	Compound CRC IC_2 / CsA CRC IC_2	Compound cell count IC_{50} / CsA cell count IC_{50}
CsA	1.0	1.0
001	1.2	13.6
009	0.5	13.6
018	0.3	3.8
020	0.4	
036		13.6
044	0.3	

045	0.3	3.1
049	0.8	12.5
050	0.5	13.6
051	0.5	4.3
052	0.7	13.6
055	0.3	13.6
056	0.3	3.1
057	0.4	13.6
058		13.6
062	0.4	13.6
064	0.2	9.6
065	0.3	8.8
067	1.7	5.9
070	0.1	9.1
071		5.2
073	0.5	13.6

Table 1

Conclusion:

Table 1 shows that the selected compounds according to the invention have a MPTP inhibitory activity at least equal to half of the CsA activity as well as at least equal to half of the activity of the compounds of the prior art. In addition the selected compounds of the invention are at least three-fold less cytotoxic than CsA as well as least three-fold less cytotoxic than the compounds of the prior art.

Example 3: **Acute pancreatitis**

Models

The compounds have been shown to significantly ameliorate disease outcome in the bile acid-induced murine acute pancreatitis model which closely reproduce clinical pathologies.

The bile acid-induced murine acute pancreatitis model is similar to gallstone induced clinical acute pancreatitis, which results from retrograde passage of bile into the pancreatic duct when gallstones impact at the sphincter of Oddi. Gallstone pancreatitis is the commonest form of the disease within the majority of countries in the world, except those where alcohol consumption is relatively very high. Biliary pancreatitis was induced by retrograde perfusion of the pancreatic duct with 2 mM TLCS; sacrifice and assessments were made 24 hours later (Laukkarinen, J.M. et al, 2007).

Results:

Single administration of 10 mg/kg compound 057 given by intraperitoneal injection one hour after pancreatic ductal infusion of bile acid abolished or markedly reduced histopathological changes, plasma amylase and interleukin-6, pancreatic myeloperoxidase and histopathology scores as represented in Figure 1.

The bile acid-induced murine acute pancreatitis model is a close representative of clinical acute pancreatitis, underlining the possible clinical utility of an early administration of compound 057 as a treatment for this disease.

List of Abbreviations

Abu	L-α-amino-*n*-butyric acid
Ac	acetyl
AcOEt	ethyl acetate
AcOH	acetic acid
Ala	L-alanine
Boc	*tert*-butyloxycarbonyl
BSEP	bile salt export pump
t-Bu	*tert*-butyl
$Ca(MeO)_2$	calcium methoxide
CC	column chromatography
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMSO	dimethyl sulfoxide
Eq	equivalent
ESI-MS	electrospray ionization mass spectrometry
EtVal	*N*-ethyl-L-valine
Fmoc	9-fluorenylmethoxycarbonyl
HATU	*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b]pyridine-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide
D-Hiv	D-hydroxyisovaleric acid
g	gramme
HCOOH	formic acid
HPLC	high performance liquid chromatography
HR-MALDI-MS	high resolution MALDI-TOF mass spectrometry
HR-Q-TOF-MS	high resolution Q-TOF mass spectrometry
Im_2CO	N,N-carbonyl-dimidazole (CDI)
Kg	kilogramme
KOMe	potassium methoxide
L	liter

MALDI	matrix-assisted laser desorption/ionization
MALDI-TOF	time-of-flight mass spectrometry
Me	methyl
D-MeAla	*N*-methyl-D-alanine
MeBmt	*N*-methyl-(4*R*)-4-[(*E*)-2-butenyl]-4,4-dimethyl-L-threonine
MeLeu	*N*-methyl-L-leucine
MeCN	acetonitrile
MeOH	methanol
MeVal	*N*-methyl-L-valine
mg	milligramme
mL	milliliter
MRP2	multidrug resistance associated protein 2
MS	mass spectrometry
MtBE	methyl-tertio-butyl-ether
NMR	nuclear magnetic resonance
NTCP	sodium-taurocholate cotransporting polypeptide
OATP1B1	organic anion transporting polypeptide 1B1
PyBOP	(benzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium-hexafluorophosphate
RP	reverse-phase
RP-HPLC	reverse-phase HPLC
RT	reaction time
Thr	L-threonine
TFA	trifluoroacetic acid
t_R	retention time
THF	tetrahydrofuran
Tj	temperature of the jacket
TLC	thin layer chromatography
Tr	temperature into the reactor
UPLC	ultra performance liquid chromatography
Vol.	volumes (1g of key raw material means 1 volume)

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Claims

1. A cycloundecadepsipeptide compound of Formula (I):

Cyclo-(AXX_1-AXX_2-AXX_3-AXX_4-AXX_5-AXX_6-AXX_7-D-Hiv-MeLeu-Leu-MeVal) (I)

1 2 3 4 5 6 7 8 9 10 11

in which:

AXX_1 is MeBmt, 4-fluoro-MeBmt, dihydro-MeBmt, 8-hydroxy-MeBmt; O-acetyl-MeBmt;

AXX_2 is Abu, Val, Thr, Thr(OMe), Thr(OAc), Thr($OCOCH_2CH_2CH_2OH$), Nva, 5-hydroxy-Nva;

AXX_3 is D-MeAla, D-3-fluoro-MeAla, D-MeSer, D-MeSer(OAc), D-MeSer(OCH_2CH_2OH), D-MeSer($OCH_2CH_2NEt_2$), D-MeAsp(OMe);

AXX_4 is MeIle, MeMet, MeVal, MeThr, MeThr(OAc), MeAla, EtVal, EtIle, EtPhe, EtTyr, EtThr(OAc), MeThr(OAc), MeTyr, MeTyr(OAc), MeTyr(OMe), MePhe, MeMet(Ox) wherein the sulphur atom of methionine is sulphoxyde or sulphone;

AXX_5 is Leu, Val, Ile, Gly, Abu;

AXX_6 is MeAla, Sar, MeLeu; and

AXX_7 is Gly, Ala;

for use in a method for treating and/or preventing cell death associated disorders or diseases, related to the MPTP opening,

and wherein said cycloundecadepsipeptide compound of Formula (I) comply with the two following criteria:

(1) MPTP inhibitory activity at least equal to half of the CsA activity, defined as:

$$\frac{Compound\_of\_formula(I)\_CRC\_IC_2}{CsA\_CRC\_IC_2} \leq 2$$

(2) at least three-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 2$$

2. The cycloundecadepsipeptide compound of claim 1, wherein said cycloundecadepsipeptide compound of Formula (I) is at least ten-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 10$$

3. The cycloundecadepsipeptide compound according to any of claims 1-2, characterised in that, in Formula (I),

AXX_1 is MeBmt

AXX_2 is Abu, Val;

AXX_3 is D-MeAla;

AXX_4 is MeIle, MeVal, EtVal ;

AXX_5 is Leu, Val, Ile, Gly, Abu;

AXX_6 is MeAla, Sar, MeLeu; and

AXX_7 is Gly, Ala.

4. The cycloundecadepsipeptide compound according to any of claims 1-3, wherein said compound is selected from the group comprising Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal), and Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal).

5. The cycloundecadepsipeptide compound according to any of claims 1-4, wherein the cell death associated disorders or diseases, related to the MPTP opening, are selected from the group comprising ischaemia-reperfusion injuries, metabolic and/or oxidative stress disorders, diseases involving loss of cellular calcium homeostasis, age related cellular degeneration, diseases involving toxic and/or infectious pathogens.

6. The cycloundecadepsipeptide compound according to any of claims 1-5, wherein the cell death associated disorders or diseases, related to the MPTP opening, are selected from the group comprising ischaemia and ischaemia-reperfusion injuries, muscular dysthrophies, neurodegeneration disorders and diseases, West Nile viral encephalitis, Japanese encephalitis, Chronic hepatitis and cirrhosis induced by Hepatitis C virus, Hepatitis B, delta agent, acute and chronic drug- or toxin-induced hepatotoxicity, alcoholic liver disease, cholestasis, Non Alcoholic Fatty Liver Disease, gastro-intestinal ulcerations caused by NSAIDs, transplant rejection, peripheral vascular insufficiency, renal insufficiency, obesity, diabetic micro-angiopathy, diabetic nephropathy, Maturity onset diabetes of the young type 4, acute and chronic pancreatitis, septicaemia, multi organ dysfunction syndrome, crush syndrome, congestive heart failure, atherosclerosis, hypertensive heart disease, cancer, bipolar disorder.

7. The cycloundecadepsipeptide compound according to claim 6, wherein ischaemia and ischaemia-reperfusion injuries are selected from the group comprising acute cerebral ischemia, acute stroke, acute myocardial infarction cardiac arrest, myocardial stunning, post-reperfusion arrhythmias, organ failure following therapeutic procedures such as thrombolytic therapy, coronary angioplasty, aortic cross-clamping or coronary bypass surgery, hypovolemic shock, storage/reperfusion injury of transplant organs, multi organ dysfunction syndrome.

8. The cycloundecadepsipeptide compound according to claim 6, wherein muscular dysthrophies are selected from the group comprising Ulrich congenital muscular dysthrophy, Bethlem myopathy, myosclerosis, limb girdle muscular dysthropy, Duchenne muscular dysthrophy, Becker muscular dysthrophy, Emery-Dreifuss syndrome,

9. The cycloundecadepsipeptide compound according to claim 6, wherein neurodegeneration disorders and diseases are selected from the group comprising traumatic brain injury, spinal cord injury, peripheral nerve injury, epilepsy-induced brain injury, amyothrophic lateral sclerosis (ALS), Alzheimer disease, Parkinson disease, multiple sclerosis, Huntington disease, axonal degeneration-induced

neuropathic pain, diabetic neuropathy chemotherapy-induced neuropathic pain, or herpes-induced neuropathy.

10. The cycloundecadepsipeptide compound according to any of claims 1-6, wherein the cell death associated disorders or diseases, related to the MPTP opening, are selected from the group comprising acute and chronic pancreatitis and muscular dystrophy.

11. A pharmaceutical composition for use in a method for treating and/or preventing cell death associated disorders and diseases, related to the MPTP opening, comprising a cycloundecadepsipeptide compound according to any of claims 1-10, together with one or more pharmaceutically acceptable diluents or carriers.

12. A method for preventing or treating cell death associated disorders or diseases, related to the MPTP opening, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of the pharmaceutical composition according to claim 11 or the cycloundecadepsipeptide compound of Formula (I):

Cyclo-(AXX$_1$-AXX$_2$-AXX$_3$-AXX$_4$-AXX$_5$-AXX$_6$-AXX$_7$-D-Hiv-MeLeu-Leu-MeVal) (I)
1 2 3 4 5 6 7 8 9 10 11

in which:

AXX$_1$ is MeBmt, 4-fluoro-MeBmt, dihydro-MeBmt, 8-hydroxy-MeBmt; O-acetyl-MeBmt;

AXX$_2$ is Abu, Val, Thr, Thr(OMe), Thr(OAc), Thr(OCOCH$_2$CH$_2$CH$_2$OH), Nva, 5-hydroxy-Nva;

AXX$_3$ is D-MeAla, D-3-fluoro-MeAla, D-MeSer, D-MeSer(OAc), D-MeSer(OCH$_2$CH$_2$OH), D-MeSer(OCH$_2$CH$_2$NEt$_2$), D-MeAsp(OMe);

AXX$_4$ is MeIle, MeMet, MeVal, MeThr, MeThr(OAc), MeAla, EtVal, EtIle, EtPhe, EtTyr, EtThr(OAc), MeThr(OAc), MeTyr, MeTyr(OAc), MeTyr(OMe), MePhe, MeMet(Ox) wherein the sulphur atom of methionine is sulphoxyde or sulphone;

AXX$_5$ is Leu, Val, Ile, Gly, Abu;

AXX_6 is MeAla, Sar, MeLeu; and

AXX_7 is Gly, Ala;

and wherein said cycloundecadepsipeptide compound of Formula (I) comply with the two following criteria:

(1) MPTP inhibitory activity at least equal to half of the CsA activity, defined as:

$$\frac{Compound\_of\_formula(I)\_CRC\_IC_2}{CsA\_CRC\_IC_2} \leq 2$$

(2) at least three-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 2$$

13. The method of claim 12, wherein said cycloundecadepsipeptide compound of Formula (I) is at least ten-fold less cytotoxic than CsA, defined as:

$$\frac{Compound\_of\_formula(I)\_cell\_count\_IC_{50}}{CsA\_cell\_count\_IC_{50}} \geq 10$$

14. The method of any of claims 12-13, wherein in Formula (I),

AXX_1 is MeBmt

AXX_2 is Abu, Val;

AXX_3 is D-MeAla;

AXX_4 is MeIle, MeVal, EtVal ;

AXX_5 is Leu, Val, Ile, Gly, Abu;

AXX_6 is MeAla, Sar, MeLeu; and

AXX_7 is Gly, Ala.

15. The method of any of claims 12-14, wherein the cycloundecadepsipeptide compound of Formula (I) is selected from the group comprising

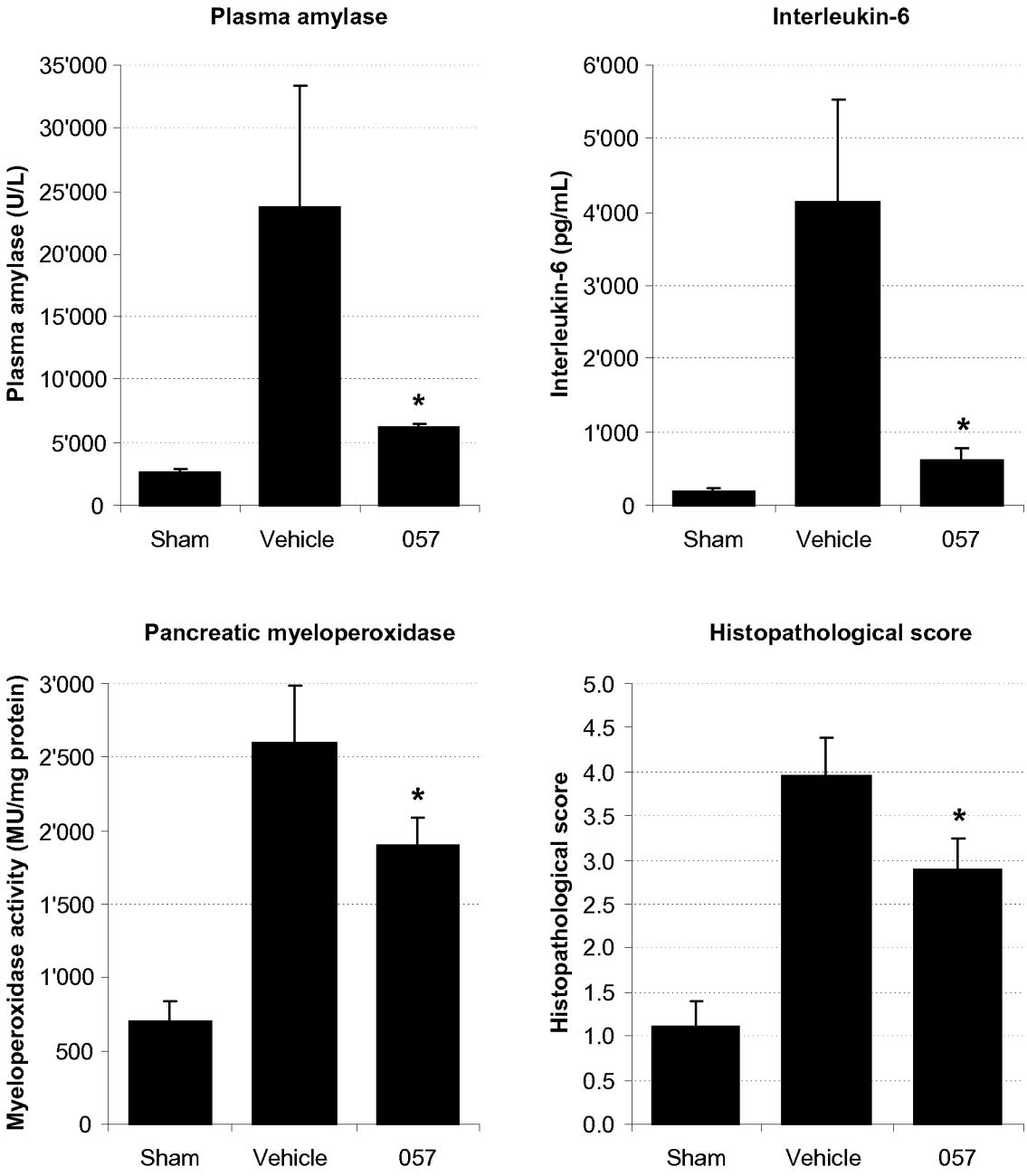
Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal), and Cyclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal)

16. The method of any of claims 12-15, wherein the cell death associated disorders or diseases, related to the MPTP opening, are selected from the group comprising ischaemia-reperfusion injuries, metabolic and/or oxidative stress disorders, diseases involving loss of cellular calcium homeostasis, age related cellular degeneration, diseases involving toxic and/or infectious pathogens.

17. The method of any of claims 12-16, wherein the cell death associated disorders or diseases, related to the MPTP opening, are selected from the group comprising ischaemia and ischaemia-reperfusion injuries, muscular dysthrophies, neurodegeneration disorders and diseases, West Nile viral encephalitis, Japanese encephalitis, Chronic hepatitis and cirrhosis induced by Hepatitis C virus, Hepatitis B, delta agent, acute and chronic drug- or toxin-induced hepatotoxicity, alcoholic liver disease, cholestasis, Non Alcoholic Fatty Liver Disease, gastro-intestinal ulcerations caused by NSAIDs, transplant rejection, peripheral vascular insufficiency, renal insufficiency, obesity, diabetic micro-angiopathy, diabetic nephropathy, Maturity onset diabetes of the young type 4, acute and chronic pancreatitis, septicaemia, multi organ dysfunction syndrome, crush syndrome, congestive heart failure, atherosclerosis, hypertensive heart disease, cancer, bipolar disorder.

18. The method of any of claims 12-17, wherein the cell death associated disorders or diseases, related to the MPTP opening, are selected from the group comprising acute and chronic pancreatitis and muscular dystrophy.

Figure 1


Plasma amylase
Plasma amylase (U/L)
35'000
30'000
25'000
20'000
15'000
10'000
5'000
0
*
Sham
Vehicle
057
Interleukin-6
Interleukin-6 (pg/mL)
6'000
5'000
4'000
3'000
2'000
1'000
0
*
Sham
Vehicle
057
Pancreatic myeloperoxidase
Myeloperoxidase activity (MU/mg protein)
3'000
2'500
2'000
1'500
1'000
500
0
*
Sham
Vehicle
057
Histopathological score
Histopathological score
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
Sham
Vehicle
057

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2011/052096

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/08 A61P9/10 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, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2006/072639 A1 (DEBIOPHARM SA [CH]; SCALFARO PIETRO [CH]; DUMONT JEAN-MAURICE [CH]; VU) 13 July 2006 (2006-07-13) claims	1-18
Y	WO 2009/098533 A1 (DEBIOPHARM SA [CH]; MOLKENTIN JEFFERY D [US]) 13 August 2009 (2009-08-13) page 4, line 6 - line 25; claims	1-18
Y	WO 02/092033 A1 (LG HOUSEHOLD & HEALTH CARE LTD [KR]; KIM SANG-NYUN [KR]; AHN HO-JEONG) 21 November 2002 (2002-11-21) page 3, line 30 - page 4, line 7 page 32, line 21 - line 24	1-18

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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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	Date of mailing of the international search report
13 July 2011	27/07/2011

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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