Novel JNK inhibitor molecules for treatment of various diseases

The present invention relates to the field of enzyme inhibition, in particular to (poly-)peptide inhibitors of c-Jun amino terminal kinase (JNK). In particular, the present invention relates to using these JNK inhibitors in the treatment of various diseases.

The c-Jun amino terminal kinase (JNK) is a member of the stress-activated group of mitogen-activated protein (MAP) kinases. These kinases have been implicated in the control of cell growth and differentiation, and, more generally, in the response of cells to environmental stimuli. The JNK signal transduction pathway is activated in response to environmental stress and by the engagement of several classes of cell surface receptors. These receptors can include cytokine receptors, serpentine receptors and receptor tyrosine kinases. In mammalian cells, JNK has been implicated in biological processes such as oncogenic transformation and mediating adaptive responses to environmental stress. JNK has also been associated with modulating immune responses, including maturation and differentiation of immune cells, as well as effecting programmed cell death in cells identified for destruction by the immune system. This unique property makes JNK signaling a promising target for developing pharmacological intervention. Among several neurological disorders, JNK signaling is particularly implicated in ischemic stroke and Parkinson's disease, but also in other diseases as mentioned further below. Furthermore, the mitogen-activated protein kinase (MAPK) p38alpha was shown to negatively regulate the cell proliferation by antagonizing the JNK-c-Jun-pathway. The mitogen-activated protein kinase (MAPK) p38alpha therefore appears to be active in suppression of normal and cancer cell proliferation and, as a further, demonstrates the involvement of JNK in cancer diseases (see e.g. Hui *et al.*, Nature Genetics, Vol 39, No. 6, June 2007). It was also shown, that c-Jun N-terminal Kinase (JNK) is involved in neuropathic pain produced by spinal nerve ligation (SNL), wherein SNL induced a slow and persistent activation of JNK, in particular JNK1, whereas p38 mitogen-activated protein kinase activation was found in spinal microglia after SNL, which had fallen to near basal level by 21 days (Zhuang et al., The Journal of Neuroscience, March 29, 2006, 26(13):3551-3560)). In 2007 (Biochemica et Biophysica Acta, pp. 1341-1348), Johnson et al.

discussed in a review the c-Jun kinase/stress-activated pathway, the involvement of JNK signalling in diseases such as the involvement in excitotoxicity of hippocampal neurons, liver ischemia, reperfusion, neurodegenerative diseases, hearing loss, deafness, neural tube birth defects, cancer, chronic inflammatory diseases, obesity, diabetes, in particular insulin-resistant diabetes, and proposed that it is likely that selective JNK inhibitors are needed for treatment of various diseases with a high degree of specificity and lack of toxicity.

Inhibition or interruption of the JNK signalling pathway is thus a promising approach in combating disorders strongly related to JNK signalling. However, there are only a few inhibitors of the JNK signaling pathway known so far.

Inhibitors of the JNK signaling pathway as already known in the prior art include e.g. upstream kinase inhibitors (for example, CEP-1347), small chemical inhibitors of JNK (SP600125 and AS601245), which directly affect kinase activity e.g. by competing with the ATP-binding site of the protein kinase, and peptide inhibitors of the interaction between JNK and its substrates (see e.g. Kuan et al., Current Drug Targets – CNS & Neurological Disorders, February 2005, vol. 4, no. 1, pp. 63-67; WO 2007/031280; all incorporated herewith by reference). WO 2007/031280 discloses small cell permeable fusion peptides, comprising a so-called TAT transporter sequence derived from the basic trafficking sequence of the HIV-TAT protein and an amino acid inhibitory sequence of IB1.

WO 2007/031280 discloses in particular two specific sequences, L-TAT-IB1 (GRKKRRQRRRPPRPKRPTTLNLFPQVPRSQD, herein SEQ ID NO: 196) and D-TAT-IB1 (dqsrpvqpflnlttprkprpprrrqrrkkrg; herein SEQ ID NO: 197), the latter being the retro-inverso sequence of L-TAT-IB1. Due to the HIV TAT derived transporter sequence, these fusion peptides are more efficiently transported into the target cells, where they remain effective until proteolytic degradation.

Since ATP independent peptide inhibitors of JNK are usually more specific inhibitors, they are frequently the first choice if it comes to inhibiting JNK. However, even the peptide inhibitors disclosed in WO 2007/031280 are not optimal for all purposes. For example, compound L-TAT-IB1 (herein SEQ ID NO: 196) which consists of L amino acids only, is quickly proteolytically degraded. In order to overcome this problem the inventors of WO 2007/031280 also suggested D-TAT-IB1 (herein SEQ ID NO: 197), which comprises D amino acids. To be more precise, D-TAT-IB1 exhibits the retro-inverso sequence of L-TAT-IB1. Incorporation of D-amino acids is made difficult by the fact that the change in stereochemistry may lead to a loss of function. The retro-

inverso approach may be employed to reduce said risk because the use of i) only D-amino acids ii) but in the inverse peptide sequence may more likely yield an acceptable conformational analogue to the original peptide than incorporating one or more D-amino acids into the original sequence. In the case of WO 2007/031280 this approach resulted nevertheless in a significant decrease in inhibitory capacity in comparison to L-TAT-IB1 (see Fig. 4). Additionally, the retro-inverso peptide is extremely stable towards proteolytic digestion with the consequence that controlled digestions, for example in time sensitive experiments, are hardly possible.

JNK inhibitors have been discussed, proposed and successfully tested in the art as treatment for a variety of disease states. Already in 1997, Dickens et al. described the c-Jun amino terminal kinase inhibitor JIP-1 and proposed JIP-1 as candidate compounds for therapeutic strategies for the treatment of for example chronic myeloid leukaemia, in particular, in the context of Bcr-Abl caused transformation of pre-B-cells (Science; 1997; 277(5326):693-696).

In 2001, Bonny and co-workers published that cell-permeable peptide inhibitors of JNK confirm long term protection to pancreatic β-cells from IL-1β-induced apoptosis and may, thus, preserve β-cells in the autoimmune destruction in the course of diabetes (Diabetes, 50, 2001, p. 77 – 82).

Bonny et al. (Reviews in Neurosciences, 2005, p. 57 – 67) discussed also the inhibitory action of the JNK inhibitor D-JNKI-1 and other JNK inhibitors in the context of excitotoxicity, neuronal cell death, hypoxia, ischemia, traumatic brain damage, epilepsy, neurodegenerative diseases, apoptosis of neurons and inner ear sensory auditory cells etc.

In WO 98/49188 JIP-1 derived inhibitors of JNK signalling are proposed for the treatment of neurodegenerative diseases, such as Parkinson's disease or Alzheimer's disease; stroke and associated memory loss, autoimmune diseases such as arthritis; other conditions characterized by inflammation; malignancies, such as leukemias, e.g. chronic myelogenous leukemia (CML); oxidative damage to organs such as the liver and kidney; heart diseases; and transplant rejections.

Borsello et al. (Nat Med, 2003, (9), p. 1180 – 1186) published that a peptide inhibitor of c-Jun-N-terminal kinase protects against excitotoxicity and cerebral ischemia.

Assi et al. have published that another specific JNK-inhibitor, SP600125, targets tumor necrosis factor-α production and epithelial cell apoptosis in acute murine colitis. The authors concluded that inhibition of JNK is of value in human inflammatory bowel disease treatment (Immunology; 2006, 118(1):112-121).

In Kennedy et al. (Cell Cycle, 2003, 2(3), p. 199 – 201), the role of JNK signalling in tumor development is discussed in more detail.

Lee Yong Hee et al. (J Biol Chem 2003, 278(5), P. 2896 – 2902) showed that c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signalling cascade and have proposed that inhibition of JNK signalling is a good therapeutic approach to reduce insulin resistance in diabetic patients.

Milano et al. (Am J Physiol Heart Circ Physiol 2007; 192(4): H1828 – H1835) discovered that a peptide inhibitor of c-Jun NH_2-terminal kinase reduces myocardial ischemia-reperfusion injury and infarct size in vivo. The authors of said study used a peptide inhibitor, D-JNKI-I, a two domain peptide containing a 20 amino acid sequence of the minimal JNK-binding domain of islet-brain-1/JNK-interacting protein-1, linked to a 10 amino acid TAT sequence of the human immuno deficiency virus TAT protein that mediates intracellular translocation. The authors have concluded that a reduction in JNK activity and phosphorylation due to the presence of said inhibitor is important in the preservation of cardiac function in rats in the phase of ischemia and apoptosis.

A further group has published that small peptide inhibitors of JNKs protect against MPTP-induced nigral dopaminergic injury via inhibiting the JNK-signalling pathway (Pan et al., Laboratory investigation, 2010, 90, 156 – 167). The authors concluded that a peptide comprising residues 153 – 163 of murine JIP-1 fused to TAT peptide offers neuroprotection against MPTP injury via inhibiting the JNK-signalling pathway and provides a therapeutic approach for Parkinson's disease.

For hearing damage, Pirvola et al. (The Journal of Neuroscience, 2000, 20(1); 43 – 50) described the rescue of hearing, auditory hair cells and neurons by CEP-1347/KT7515, an inhibitor of c-Jun-N-terminal kinase activation. The authors suggested in general that therapeutic intervention in the JNK signalling cascade may offer opportunities to treat inner ear injuries. Treatment of hearing loss by means of administering JNK-inhibitory peptides is also disclosed for example in WO 03/103698.

For retinal diseases and age-related macula degeneration in particular, Roduit et al. (Apoptosis, 2008, 13(3), p. 343 – 353) have likewise suggested to use JNK-inhibition as therapeutic approach. Similar considerations relying on JNK-inhibition are disclosed for example in WO 2010/113753 for the treatment of age-related macular degeneration, diabetic macular edema, diabetic retinopathy, central exudative chorioretinopathy, angioid streaks, retinal pigment epithelium

detachment, multifocal choroiditis, neovascular maculopathy, retinopathy of prematurity, retinitis pigmentosa, Leber's disease, retinal artery occlusion, retinal vein occlusion, central serous chorioretinopathy, retinal macroaneurysm, retinal detachment, proliferative vitreoretinopathy, Stargardt's disease, choroidal sclerosis, chorioderemia, vitelliform macular dystrophy, Oguchi's disease, fundus albipunctatus, retinitis punctata albescens, and gyrate atrophy of choroid and retina.

Zoukhri et al. (Journal of Neurochemistry, 2006, 96, 126 – 135) identified that c-Jun NH_2-terminal kinease mediates interleukin-1 β-induced inhibition of lacrimal gland secretion. They concluded that JNK plays a pivotal role in IL-1 β-mediated inhibition of lacrimal gland secretion and subsequent dry eye.

For uveitis, Touchard et al. (Invest Ophthalmol Vis Sci, 2010, 51(9); 4683 – 4693) have suggested to use D-JNKI 1 as effective treatment.

For IBD (inflammatory bowel disease) Roy et al. (World J Gastroenterol 2008, 14(2), 200 – 202) have highlighted the role of the JNK signal transduction pathway therein and have proposed to use peptidic JNK inhibitors for the treatment of said disease state.

Beckham et al (J Virol. 2007 Jul;81(13):6984-6992) showed that the JNK inhibitor D-JNKI-1 is effective in protecting mice from viral encephalitis, and suggest thus JNK inhibition as promising and novel treatment strategy for viral encephalitis.

Palin et al. (Psychopharmacology (Berl). 2008 May;197(4):629-635) used the same JNK inhibitor, D-JNKI-1, and found that pre-treatment with D-JNKI-1 (10 ng/mouse), but not D-TAT, significantly inhibited all three indices of sickness induced by central TNFalpha and suggested that JNK inhibition as means for treating major depressive disorders that develop on a background of cytokine-induced sickness behaviour.

In WO 2010/151638 treatment of the neurodegenerative disease spinal muscular atrophy by way of JNK inhibition was proposed.

The above passage highlights already on basis of only some selected publications the usefulness of JNK inhibitors in the treatment of various diseases. Thus, there is a constant need in the art for JNK inhibitors for use in the treatment of human (and animal) diseases.

Thus, the problem to be solved by the present invention was to provide further (peptide) inhibitors of JNK.

The object of the present invention is solved by the inventor by means of the subject-matter set out below and in the appended claims.

Brief description of the Figures

In the following a brief description of the appended figures will be given. The figures are intended to illustrate the present invention in more detail. However, they are not intended to limit the subject matter of the invention in any way.

Fig. 1: Illustration of the inhibitory efficacy of several JNK inhibitors according to the present invention, which was investigated by in vitro AlphaScreen assay (Amplified Luminescence Proximity Homogeneous-Screen Assay).
Fig.1A: Inhibition of JNK1 by SEQ ID NOs: 193, 2, 3, 5, 6, and 7.
Fig.1B: Inhibition of JNK2 by SEQ ID NOs: 193, 2, 3, 5, 6, and 7.
Fig.1C: Inhibition of JNK3 by SEQ ID NOs: 193, 2, 3, 5, 6, and 7.

Fig. 2: Table illustrating the inhibitory efficacy of several JNK inhibitors (SEQ ID NOs: 193, 2, 3, 5, 6, and 7) according to the present invention. Given are the IC50 values in the nM range, the respective standard error of the mean and the number of experiments performed (n).

Fig. 3: Illustration of the inhibitory efficacy of several JNK inhibitors according to the present invention, which are fusion proteins of a JNK inhibitory (poly-)peptide sequence and a transporter sequence. The inhibitory efficacy was determined by means of in vitro AlphaScreen assay (Amplified Luminescence Proximity Homogeneous-Screen Assay).
Fig.3A: Inhibition of JNK1 by SEQ ID NOs: 194, 195, 172, 200, 46, 173, 174, 175, 176, 177, 178, 179, 180, 181 and 197.
Fig.3B: Inhibition of JNK2 by SEQ ID NOs: 194, 195, 172, 200, 46, 173, 174, 175, 176, 177, 178, 179, 180, 181 and 197.
Fig.3C: Inhibition of JNK3 by SEQ ID NOs: 194, 195, 172, 200, 46, 173, 174, 175, 176, 177, 178, 179, 180, 181 and 197.
Fig.3D: Inhibition of JNK1 by SEQ ID NOs: 194, 195, 172, 200, 46, 182, 183, 184, 185, 186, 187, 188, 189, 190 and 197.

Fig.3E: Inhibition of JNK2 by SEQ ID NOs: 194, 195, 172, 200, 46, 182, 183, 184, 185, 186, 187, 188, 189, 190 and 197.

Fig.3F: Inhibition of JNK3 by SEQ ID NOs: 194, 195, 172, 200, 46, 182, 183, 184, 185, 186, 187, 188, 189, 190 and 197.

Fig. 4: Table illustrating the inhibitory efficacy of several JNK inhibitors according to the present invention, which are fusion proteins of a JNK inhibitory (poly-)peptide sequence and a transporter sequence. Given are the IC50 values in the nM range, the respective standard error of the mean (SEM) and the number of experiments performed (n).

Fig. 5: Stability of JNK inhibitors with SEQ ID NOs: 172, 196 and 197 in 50% human serum. The JNK inhibitor with SEQ ID NO: 196 was totally degraded into amino acids residues within 6 hours (A). The JNK inhibitor with SEQ ID NO: 172 was completely degraded only after 14 days (B). The JNK inhibitor with SEQ ID NO: 197 was stable at least up to 30 days (B).

Fig. 6: shows internalizations experiments using TAT derived transporter constructs with D-amino acid/L-amino acid pattern as denoted in SEQ ID NO: 30. The transporter sequences analyzed correspond to SEQ ID NOs: 52-94 plus SEQ ID NOs: 45, 47, 46, 43 and 99 (Fig 6a) and SEQ ID NOs: 100-147 (Fig. 6b). As can be seen, all transporters with the consensus sequence rXXXrXXXr (SEQ ID NO: 31) showed a higher internalization capability than the L-TAT transporter (SEQ ID NO: 43). Hela cells were incubated 24 hours in 96well plate with 10mM of the respective transporters. The cells were then washed twice with an acidic buffer (0.2M Glycin, 0.15M NaCl, pH 3.0) and twice with PBS. Cells were broken by the addition of RIPA lysis buffer. The relative amount of internalized peptide was then determined by reading the fluorescence intensity (Fusion Alpha plate reader; PerkinElmer) of each extract followed by background subtraction.

Fig. 7 The JNK inhibitor with the sequence of SEQ ID NO: 172 blocks LPS-induced cytokine and chemokine release in THP1- PMA-differentiated macrophages. Fig. 7A: TNF release (THP1pma 6h 3ng/ml LPS); Fig. 7B: TNFa release (THP1pma 6h 10ng/ml LPS); Fig. 7C: IL 6 release (THP1pma 6h 10ng/ml LPS); Fig. 7D: MCP1 release (THP1pma 6h 3ng/ml LPS).

Fig. 8 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced IL6 release in THP1 differentiated macrophages with higher potency than D-TAT-IB1 (SEQ ID NO: 197), dTAT (SEQ ID NO: 45) and SP 600125. LPS was added for 6h (10 ng/ml).

Fig. 9 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced TNFα release in THP1 differentiated macrophages with higher potency than D-TAT-IB1 (SEQ ID NO: 197), dTAT (SEQ ID NO: 45) and SP 600125. LPS was added for 6h (10 ng/ml).

Fig. 10 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced IL-6 release in PMA differentiated macrophages with higher potency than D-TAT-IB1 (SEQ ID NO: 197) and L-TAT-IB1 (SEQ ID NO: 196). LPS was added for 6h.

Fig. 11 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced TNFα release in PMA differentiated macrophages with higher potency than D-TAT-IB1 (SEQ ID NO: 197) and L-TAT-IB1 (SEQ ID NO: 196).

Fig. 12 The JNK inhibitor of SEQ ID NO: 172 blocks LPS-induced TNFα release in Primary Rat Whole Blood Cells at 3 ng/ml. Given are the results for the control, 1 μM of SEQ ID NO: 172, 3 μM of SEQ ID NO: 172, and 10 μM of SEQ ID NO: 172 at different levels of LPS (ng/ml).

Fig. 13 The JNK inhibitor of SEQ ID NO: 172 blocks IL2 secretion by primary human T-cells in response to PMA/Ionomycin.

Fig. 14 The JNK inhibitor of SEQ ID NO: 172 blocks IL2 secretion by primary human T-cells in response to CD3/CD28 stimulation. The JNK inhibitors used are indicated by their SEQ ID NO: 172 and 197.

Fig. 15 Dose-dependent inhibition by JNK inhibitor with SEQ ID NO: 172 of CD3/CD28-induced IL-2 release in primary rat lymph-nodes purified T cells. Control rat were sacrificed and lymph-nodes were harvested. T cells further were purified (using magnetic negative selection) and plated into 96-well plates at 200.000 cells/well. Cells were treated with anti-rat CD3 and anti-rat CD28 antibodies (2μg/mL). JNK inhibitor with SEQ ID NO: 172 was added to the cultures 1h before CD3/CD28 treatment and IL-2 release was assessed in supernatant 24h after treatment.

Fig. 16 Dose-dependent inhibition of CD3/CD28-induced IL-2 release in primary rat lymph-nodes purified T cells: Comparison of several JNK inhibitors, namely SEQ ID NOs: 172, 197 and SP600125.

Fig. 17 Dose dependent inhibition of IL-2 release in rat whole blood stimulated with PMA + ionomycin. JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10 µM 1h before stimulation with PMA + ionomycin. Three doses of activators were added (25/500 ng/mL, 50/750 ng/mL and 50/1000 ng/mL) for 4h. IL-2 release was assessed in supernatant. JNK inhibitor with SEQ ID NO: 172 at 10µM did efficiently reduce PMA-iono-induced IL-2 release at the three tested activator concentrations.

Fig. 18 JNK inhibition and IL-6 release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10µM 1h before whole blood stimulation with LPS (0.02ng/mL) for 4 hours. The JNK inhibitor with SEQ ID NO: 172 did reduce the LPS-induced IL-6 release in a dose-dependent manner.

Fig. 19 JNK inhibition and IL-2 release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10µM 1h before whole blood stimulation with PMA+ionomycin (25/700ng/mL, 50/800ng/ml and 50/1000ng/mL) for 4 hours. The JNK inhibitor with SEQ ID NO: 172 did reduce the PMA+ionomycin -induced IL-2 release in a dose-dependent manner.

Fig. 20 JNK inhibition and IFN-γ release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10µM 1h before whole blood stimulation with PMA+ionomycin (25/700ng/mL, 50/800ng/ml and 50/1000ng/mL) for 4 hours. The JNK inhibitor with SEQ ID NO: 172 did reduce the PMA+ionomycin -induced IFN-γ release in a dose-dependent manner.

Fig. 21 JNK inhibition and TNF-α release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10µM 1h before whole blood stimulation with PMA+ionomycin (25/700ng/mL, 50/800ng/ml and 50/1000ng/mL) for 4 hours. The JNK inhibitor with SEQ ID NO: 172 did reduce the PMA+ionomycin -induced TNF-α release in a dose-dependent manner.

Fig. 22 JNK inhibition and TNF-α release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10µM 1h before whole blood stimulation with PHA-L (5µg/mL) for 3 days. The JNK inhibitor with SEQ ID NO: 172 did reduce the PHA-L-induced TNF-α release in a dose-dependent manner.

Fig. 23 JNK inhibition and IL-2 release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10μM 1h before whole blood stimulation with PHA-L (5μg/mL) for 3 days. The JNK inhibitor with SEQ ID NO: 172 did reduce the PHA-L-induced IL-2 release in a dose-dependent manner.

Fig. 24 JNK inhibition and TNF-α release in human whole blood. The JNK inhibitor with SEQ ID NO: 172 was added at three different concentrations, namely 1, 3 and 10μM 1h before whole blood stimulation with CD3 +/- CD28 antibodies (2μg/mL) for 3 days. The JNK inhibitor with SEQ ID NO: 172 did reduce the CD3/CD28-induced TNF-α release in a dose-dependent manner.

Fig. 25 Photograhic illustration of in vivo anti-inflammatory properties of the JNK inhibitors with SEQ ID NO: 197 (10 μg/kg) and SEQ ID NO: 172 (10 μg/kg) after CFA (complete Freund's adjuvant) induced paw swelling. Paw swelling was induced in the left hind paw, the right hind paw was not treated.

Fig. 26 Graphical representation of in vivo anti-inflammatory properties of the JNK inhibitors with SEQ ID NO: 197 (10 μg/kg, n=4) and SEQ ID NO: 172 (10 μg/kg, n=3) after CFA (complete Freund's adjuvant) induced paw swelling. Indicated is the measured circumference of the left hind paw after treatment.

Fig. 27 Graphical representation of in vivo anti-inflammatory properties of the JNK inhibitors with SEQ ID NO: 197 (10 μg/kg) and SEQ ID NO: 172 (10 μg/kg) after CFA (complete Freund's adjuvant) induced paw swelling. Indicated is the measured in vivo cytokine release one hour after CFA induced paw swelling.

Fig. 28 Clinical evaluation of administration of different amounts of the JNK inhibitor according to SEQ ID NO: 172 in albino rats after intravenous administration (endotoxins induced uveitis model). Form left to right: Vehicle, 0.015 mg/kg (i.v.) of SEQ ID NO: 172; 0.18 mg/kg (i.v.) of SEQ ID NO: 172; 1.8 mg/kg (i.v.) of SEQ ID NO: 172, 2 mg/kg (i.v.) of SEQ ID NO: 197 and 20 μg dexamethasone (administered directly by subconjunctival injection to the eye). Indicated is the clinical score (mean and the SEM).

Fig. 29 Responsive effects of the JNK inhibitor of SEQ ID NO: 172 after daily intravenous administration in 14 day rat chronic established Type II collagen arthritis (RTTC/SOL-1). Shown is the body weight change from day 0 to day 14. From left to right: Normal contrail

+ Vehicle (NaCl), Disease Control + Vehicle (NaCl), 5 mg/kg (i.v.) of SEQ ID NO: 172; 1 mg/kg (i.v.) of SEQ ID NO: 172; 0.1 mg/kg (i.v.) of SEQ ID NO: 172, 0.01 mg/kg (i.v.) of SEQ ID NO: 172, 0.05 mg/kg (i.v.) of dexamethasone. Indicated is the clinical score (mean and the SEM). n= 4/normal group, n=8/treatment group; *p ≤0.05 1-way ANOVA to disease control + Vehicle (NaCl)

Fig.30 Responsive effects of the JNK inhibitor of SEQ ID NO: 172 after daily intravenous administration in 14 day rat chronic established Type II collagen arthritis (RTTC/SOL-1). Shown is the ankle diameter (in) over time. n= 4/normal group, n=8/treatment group; *p ≤0.05 2-way RM ANOVA to disease control + Vehicle (NaCl).

Fig. 31 Responsive effects of the JNK inhibitor of SEQ ID NO: 172 after daily intravenous administration in 14 day rat chronic established Type II collagen arthritis (RTTC/SOL-1). Illustrated are the ankle histopathology scores regarding inflammation, pannus, cartilage damage and bone resorption. n=8 in the treatment group. *p ≤0.05 Mann-Whitney U test to disease control + Vehicle (NaCl).

Fig. 32 Responsive effects of the JNK inhibitor of SEQ ID NO: 172 after daily intravenous administration in 14 day rat chronic established Type II collagen arthritis (RTTC/SOL-1). Illustrated are the knee histopathology scores regarding inflammation, pannus, cartilage damage and bone resorption. n=8 in the treatment group. *p ≤0.05 Mann-Whitney U test to disease control + Vehicle (NaCl).

Fig. 33 Clinical scoring by slit lamp 24 hours after EIU induction and administration of JNK inhibitor according to SEQ ID NO: 172 (1 mg/kg i.v.) at different times prior to EIU induction. From left to right: Vehicle (0 hours); SEQ ID NO: 172 4 weeks prior to EIU induction; SEQ ID NO: 172 2 weeks prior to EIU induction; SEQ ID NO: 172 1 week prior to EIU induction; SEQ ID NO: 172 48 hours prior to EIU induction; SEQ ID NO: 172 24 hours prior to EIU induction; SEQ ID NO: 172 0 hours prior to EIU induction; Dexamethasone (2 mg/kg i.v.) 0 hours prior to EIU induction. Mean ± SEM. *p<0.05 versus vehicle, **p<0.01 versus vehicle.

Fig. 34 Number of PMN cells per section quantified 24 hours after EIU induction and administration of JNK inhibitor according to SEQ ID NO: 172 (1 mg/kg i.v.) at different times prior to EIU induction. From left to right: Vehicle (0 hours); SEQ ID NO: 172 4 weeks prior to EIU induction; SEQ ID NO: 172 2 weeks prior to EIU induction; SEQ ID NO: 172

1 week prior to EIU induction; SEQ ID NO: 172 48 hours prior to EIU induction; SEQ ID NO: 172 24 hours prior to EIU induction; SEQ ID NO: 172 0 hours prior to EIU induction; Dexamethasone (2 mg/kg i.v.) 0 hours prior to EIU induction. Mean ± SEM. *p<0.05 versus vehicle, **p<0.01 versus vehicle.

Fig. 35 shows the mean calculated TBUT AUC values for animals with scopolamine induced dry eye syndrome. Shown are the results for animals treated with vehicle, 3 different concentrations of an all-D-retro-inverso JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 197, 3 different concentrations of a JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 172, and the results for animals treated with cyclosporine.

Fig. 36 shows the mean calculated PRTT AUCs for animals with scopolamine induced Dry Eye (Day 7-21). Shown are the results for animals treated with vehicle, 3 different concentrations of an all-D-retro-inverso JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 197, 3 different concentrations of a JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 172, and the results for animals treated with cyclosporine.

Fig. 37 shows the mean histological Cornea Lesion Scores for animals with scopolamine induced dry eye syndrome. Shown are the results for animals treated with vehicle, 3 different concentrations of an all-D-retro-inverso JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 197, 3 different concentrations of a JNK-inhibitor (poly-)peptide with the sequence of SEQ ID NO: 172, and the results for animals treated with cyclosporine.

JNK inhibitors

In a first aspect the present invention relates to a JNK inhibitor, which comprises an inhibitory (poly-)peptide sequence according to the following general formula:

X1-X2-X3-R-X4-X5-X6-L-X7-L-X8 (SEQ ID NO: 1),

wherein X1 is an amino acid selected from amino acids R, P, Q and r,
wherein X2 is an amino acid selected from amino acids R, P, G and r,
wherein X3 is an amino acid selected from amino acids K, R, k and r,
wherein X4 is an amino acid selected from amino acids P and K,
wherein X5 is an amino acid selected from amino acids T, a, s, q, k or is absent,
wherein X6 is an amino acid selected from amino acids T, D and A,
wherein X7 is an amino acid selected from amino acids N, n, r and K; and

wherein X8 is an amino acid selected from F, f and w,

with the proviso that at least one, at least two, at least three, at least four, at least five or six of the amino acids selected from the group consisting of X1, X2, X3, X5, X7 and X8 is/are a D-amino acid(s), preferably with the proviso that at least one, at least two, at least three or four of the amino acids selected from the group consisting of X3, X5, X7 and X8 is/are a D-amino acid(s),

for use in a method for treatment of the human or animal body by therapy.

The inhibitory (poly-)peptide sequence of the JNK inhibitor according to the present invention comprises L-amino acids and in most embodiments D-amino acids. Unless specified otherwise, L-amino acid residues are indicated herein in capital letters, while D amino acid residues are indicated in small letters. Glycine may be indicated in capital or small letters (since there is no D- or L-glycine). The amino acid sequences disclosed herein are always given from N- to C-terminus (left to right) unless specified otherwise. The given amino acid sequence may be modified or unmodified at the C- and/or N-terminus, e.g. acetylation at the C-terminus and/or amidation or modification with cysteamide at the N-terminus. For sake of clarity such possible but entirely optional modifications at the C- and/or N-terminus of the amino acid sequences disclosed herein are for sake of clarity not specifically indicated.

The JNK inhibitors of the present invention are (poly-)peptide inhibitors of the c-Jun N-terminal kinase (JNK). Said inhibitors inhibit the kinase activity of c-Jun N-terminal kinase (JNK), i.e. prevent or reduce the extent of phosphorylation of JNK substrates such as c-Jun, ATF2 and/or Elk-1. A person skilled in the art will understand that the term "inhibitor", as used herein, does not comprise compounds which irreversibly destroy the c-Jun N-terminal kinase (JNK) molecule and/or kinase activity. Furthermore, the term "inhibiting JNK activity" as used herein, refers to the inhibition of the kinase activity of c-Jun N-terminal kinase (JNK).

Furthermore, as used herein, a JNK inhibitor comprises at least one functional unit of a polymer of amino acids, i.e. a (poly-)peptide sequence. Moreover, this at least one functional polymer of amino acids provides for inhibition of JNK activity. The amino acid monomers of said inhibitory (poly-)peptide sequence are usually linked to each other via peptide bonds, but (chemical) modifications of said peptide bond(s) or of side chain residues may be tolerable, provided the inhibitory activity (inhibition of JNK activity) is not totally lost, i.e. the resulting chemical entity still qualifies as JNK inhibitor as functionally defined herein. The term "(poly-)peptide" shall not be

construed as limiting the length of the (poly-)peptide unit. Preferably, the inhibitory (poly-)peptide sequence of the JNK inhibitors of the present invention is less than 500, 490, 480, 470, 460, 450, 440, 430, 420, 410, 400, 390, 380, 370, 360, 350, 340, 330, 320, 310, 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, or less than 12 amino acids long. Preferably, the inhibitory (poly-)peptide sequence does not have less than 10 amino acid residues, more preferably not less than 11 amino acid residues.

Furthermore, a "JNK inhibitor" of the present invention inhibits JNK activity, e.g. exhibits with regard to the inhibition of human JNK mediated phosphorylation of a c-Jun substrate (SEQ ID NO: 198) an IC 50 value of:

a) less than 3000 nM, more preferably less than 2000 nM, even more preferably less than 1000 nM, even more preferably less than 500 nM, even more preferably less than 250 nM, even more preferably less than 200 nM, even more preferably less than 150 nM, most preferably less than 100 nM with regard to inhibition of human JNK1,

b) less than 3000 nM, more preferably less than 2000 nM, even more preferably less than 1000 nM, even more preferably less than 500 nM, even more preferably less than 250 nM, even more preferably less than 200 nM, even more preferably less than 150 nM, most preferably less than 100 nM with regard to inhibition of human JNK2, and/or

c) less than 3000 nM, more preferably less than 2000 nM, even more preferably less than 1000 nM, even more preferably less than 500 nM, even more preferably less than 250 nM, even more preferably less than 200 nM, even more preferably less than 150 nM, most preferably less than 100 nM with regard to inhibition of human JNK3.

For some applications it is preferred that the inhibitor inhibits human JNK2 and/or human JNK3 according to the above definition, but not JNK1 according to the above definition.

Whether JNK activity is inhibited or not, may easily be assessed by a person skilled in the art. There are several methods know in the art. One example is a radioactive kinase assay or a non-radioactive kinase assay (e.g. Alpha screen test; see for example Guenat et al. J Biomol Screen, 2006; 11: pages 1015-1026).

A JNK inhibitor according to the present invention may thus for example comprise an inhibitory (poly-)peptide sequence according to any of SEQ ID NOs: 2 to 27 (see table 1).

Table 1:
Examples for inhibitory (poly-)peptide sequences of JNK-inhibitors according to the present invention

Amino acid sequence	SEQ ID NO:
rPKRPTTLNLF	2
RPkRPTTLNLF	3
RPKRPaTLNLF	4
RPKRPTTLnLF	5
RPKRPTTLrLF	6
RPKRPTTLNLf	7
RPkRPaTLNLf	8
RPkRPTTLNLf	9
RPkRPTTLrLf	10
RRrRPTTLNLf	11
QRrRPTTLNLf	12
RPkRPTTLNLw	13
RPkRPTDLNLf	14
RRrRPTTLrLw	15
QRrRPTTLrLw	16
RRrRPTDLrLw	17
QRrRPTDLrLw	18
RRrRPaTLNLf	19
QRrRPaTLNLf	20
RrKRPaTLNLf	21
RPkRPsTLNLf	22
RPkRPqTLNLf	23
RPkRPkTLNLf	24
rGKRKALKLf	25
rGKRKALrLf	26
RRrRKALrLf	27

The JNK inhibitor according to the present invention may also be a JNK inhibitor (variant) which comprises an inhibitory (poly-)peptide sequence sharing at least 50%, more preferably at least 55%, more preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, most preferably at least 90% sequence identity with a sequence selected from SEQ ID NOs: 1-27, in particular with SEQ ID NO: 8,

with the proviso that with regard to the respective sequence selected from SEQ ID NOs: 1-27, such inhibitory (poly-)peptide sequence sharing sequence identity

a) maintains the L-arginine (R) residue on position 4,

b) maintains the two L-leucine (L) residues at position 8 and 10 (positions 7 and 9 with regard to SEQ ID NOs: 25-27),

c) exhibits one, two, three, four, five or six D-amino acid(s) at the respective positions corresponding to the amino acids selected from the group consisting of X1, X2, X3, X5, X7 and X8 of SEQ ID NO: 1 and respective positions in SEQ ID NOs: 2-27, more preferably exhibits one, two, three or four D-amino acid(s) at the positions corresponding to the amino acids selected from the group consisting of X3, X5, X7 and X8 of SEQ ID NO: 1 and respective positions in SEQ ID NOs: 2-27, and

d) still inhibits JNK activity (i.e. is a JNK inhibitor as defined herein).

Certainly, variants disclosed herein (in particular JNK inhibitor variants comprising an inhibitory (poly-)peptide sequence sharing - within the above definition – a certain degree of sequence identity with a sequence selected from SEQ ID NOs: 1-27), share preferably less than 100% sequence identity with the respective reference sequence.

In view of said definition and for sake of clarity the residues which may not be changed in variants of JNK inhibitors comprising SEQ ID NOs: 1-27 (see a) and b) in the above definition) are underlined in table 1.

The non-identical amino acids are preferably the result of conservative amino acid substitutions.

Conservative amino acid substitutions, as used herein, may include amino acid residues within a group which have sufficiently similar physicochemical properties, so that a substitution between members of the group will preserve the biological activity of the molecule (see e.g. Grantham, R. (1974), Science *185*, 862-864). Particularly, conservative amino acid substitutions are preferably substitutions in which the amino acids originate from the same class of amino acids (e.g. basic

amino acids, acidic amino acids, polar amino acids, amino acids with aliphatic side chains, amino acids with positively or negatively charged side chains, amino acids with aromatic groups in the side chains, amino acids the side chains of which can enter into hydrogen bridges, e.g. side chains which have a hydroxyl function, etc.). Conservative substitutions are in the present case for example substituting a basic amino acid residue (Lys, Arg, His) for another basic amino acid residue (Lys, Arg, His), substituting an aliphatic amino acid residue (Gly, Ala, Val, Leu, Ile) for another aliphatic amino acid residue, substituting an aromatic amino acid residue (Phe, Tyr, Trp) for another aromatic amino acid residue, substituting threonine by serine or leucine by isoleucine. Further conservative amino acid exchanges will be known to the person skilled in the art. The isomer form should preferably be maintained, e.g. K is preferably substituted for R or H, while k is preferably substituted for r and h.

Further possible substitutions within the above definition for JNK inhibitor variants are for example if:

a) one, two or more of X1, X2, X3, X4, X5, X6, X7 and/or X8 of SEQ ID NO: 1 or the corresponding positions within the respective sequence selected from SEQ ID NOs: 2-27 are substituted for A or a,

b) X1 or X8 of SEQ ID NO: 1 or the corresponding position within the respective sequence selected from SEQ ID NOs: 2-27 is deleted;

c) X5 of SEQ ID NO: 1 or the corresponding position within the respective sequence selected from SEQ ID NOs: 2-27 is E, Y, L, V, F or K;

d) X5 of SEQ ID NO: 1 or the corresponding position within the respective sequence selected from SEQ ID NOs: 2-27 is E, L, V, F or K; or

e) one, two or three of X1, X2, X3 of SEQ ID NO: 1 or the corresponding positions within the respective sequence selected from SEQ ID NOs: 2-27 are neutral amino acids.

As used herein, the term "% sequence identity", has to be understood as follows: Two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may then be determined over the whole length of each of the sequences being compared (so-called global alignment), that is particularly suitable for sequences of the same or similar length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length. In the above context, an amino acid sequence having a "sequence identity" of at least, for example, 95% to a query amino acid sequence, is intended to mean that the sequence of the subject amino acid sequence is identical to the query sequence except that

the subject amino acid sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain an amino acid sequence having a sequence of at least 95% identity to a query amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the subject sequence may be inserted or substituted with another amino acid or deleted. For purposes of determining sequence identity, the substitution of an L-amino acid for a D-amino acid (and vice versa) is considered to yield a non-identical residue, even if it is merely the D- (or L-isomer) of the very same amino acid.

Methods for comparing the identity and homology of two or more sequences are well known in the art. The percentage to which two sequences are identical can for example be determined by using a mathematical algorithm. A preferred, but not limiting, example of a mathematical algorithm which can be used is the algorithm of Karlin *et al.* (1993), PNAS USA, 90:5873-5877. Such an algorithm is integrated in the BLAST family of programs, e.g. BLAST or NBLAST program (see also Altschul *et al.*, 1990, J. Mol. Biol. 215, 403-410 or Altschul *et al.* (1997), Nucleic Acids Res, 25:3389-3402), accessible through the home page of the NCBI at world wide web site ncbi.nlm.nih.gov) and FASTA (Pearson (1990), Methods Enzymol. *183*, 63-98; Pearson and Lipman (1988), Proc. Natl. Acad. Sci. U. S. A *85*, 2444-2448.). Sequences which are identical to other sequences to a certain extent can be identified by these programmes. Furthermore, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux *et al.*, 1984, Nucleic Acids Res., 387-395), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of (Smith and Waterman (1981), J. Mol. Biol. *147*, 195-197.) and finds the best single region of similarity between two sequences.

Certainly, the JNK inhibitor according to the present invention may comprise - aside of the inhibitory (poly-)peptide sequence mentioned above – additional sequences, domains, labels (e.g. fluorescent or radioactive labels), epitopes etc. as long as the ability to inhibit JNK activity as defined herein is not lost. For example, the JNK inhibitor according to the present invention may also comprise a transporter sequence. A "transporter sequence" as used herein, is a (poly-)peptide sequence providing for translocation of the molecule it is attached to across biological membranes. Accordingly, a JNK inhibitor according to the present invention comprising a transporter sequence is preferably capable of translocating across biological membranes. Thus, such JNK inhibitor of the present invention may more readily enter a cell, a cellular subcompartiment and/or into the nucleus of a cell.

Said transporter sequence may be joined for example (e.g. directly) N-terminally or (e.g. directly) C-terminally to the inhibitory (poly-)peptide sequence of the JNK inhibitor. The transporter sequence and the inhibitory (poly-)peptide sequence may also be spaced apart, e.g. may be separated by intermediate sequences. It is also contemplated that the transporter sequence may be positioned entirely elsewhere in the JNK inhibitor molecule than the inhibitory (poly-)peptide sequence, in particular if the JNK inhibitor is a more complex molecule (e.g. comprising several domains, is a multimeric conjugate etc.). It is also contemplated that the transporter sequence and the inhibitory (poly-)peptide sequence may overlap as long as the JNK inhibitory activity is maintained. Examples for such overlap are given further below.

Transporter sequences for use with the JNK inhibitor of the present invention may be selected from, without being limited thereto, transporter sequences derived from HIV TAT (HIV), e.g. native proteins such as e.g. the TAT protein (e.g. as described in U.S. Patent Nos. 5,804,604 and 5,674,980, each of these references being incorporated herein by reference), HSV VP22 (*Herpes simplex*) (described in e.g. WO 97/05265; Elliott and O'Hare, Cell 88 : 223-233 (1997)), non-viral proteins (Jackson et al, Proc. Natl. Acad. Sci. USA 89 : 10691-10695 (1992)), transporter sequences derived from Antennapedia, particularly from *Drosophila antennapedia* (e.g. the antennapedia carrier sequence thereof), FGF, lactoferrin, etc. or derived from basic peptides, e.g. peptides having a length of 5 to 15 amino acids, preferably 10 to 12 amino acids and comprising at least 80 %, more preferably 85 % or even 90 % basic amino acids, such as e.g. arginine, lysine and/or histidine, or may be selected from e.g. arginine rich peptide sequences, such as RRRRRRRRR (R_9; SEQ ID NO: 152), RRRRRRR (R_8; SEQ ID NO: 153), RRRRRR (R_7; SEQ ID NO: 154), RRRRR (R_6, SEQ ID NO: 155), RRRR (R_5, SEQ ID NO: 156) etc., from VP22, from PTD-4 proteins or peptides, from RGD-K_{16}, from PEPT1/2 or PEPT1/2 proteins or peptides, from SynB3 or SynB3 proteins or peptides, from PC inhibitors, from P21 derived proteins or peptides, or from JNKI proteins or peptides.

Examples of transporter sequences for use in the JNK inhibitor of the present invention are in particular, without being limited thereto, basic transporter sequences derived from the HIV-1 TAT protein. Preferably, the basic transporter sequence of the HIV-1 TAT protein may include sequences from the human immunodeficiency virus HIV-1 TAT protein, e.g. as described in, e.g., U.S. Patent Nos. 5,804,604 and 5,674,980, each incorporated herein by reference. In this context, the full-length HIV-1 TAT protein has 86 amino acid residues encoded by two exons of the HIV TAT gene. TAT amino acids 1-72 are encoded by exon 1, whereas amino acids 73-86 are encoded by exon 2. The full-length TAT protein is characterized by a basic region which contains two lysines and six arginines (amino acids 49-57) and a cysteine-rich region which contains seven

cysteine residues (amino acids 22-37). The basic region (i.e., amino acids 49-57) was thought to be important for nuclear localization. Ruben, S. *et al.*, J. Virol. 63: 1-8 (1989); Hauber, J. *et al.*, J. Virol. 63 1181-1187 (1989). The cysteine-rich region mediates the formation of metal-linked dimers in vitro (Frankel, A. D. et al, Science 240: 70-73 (1988); Frankel, A. D. *et al.*, Proc. Natl. Acad. Sci USA 85: 6297-6300 (1988)) and is essential for its activity as a transactivator (Garcia, J. A. *et al.*, EMBO J. 7: 3143 (1988); Sadaie, M. R. *et al.*, J. Virol. 63:1 (1989)). As in other regulatory proteins, the N-terminal region may be involved in protection against intracellular proteases (Bachmair, A. *et al.*, Cell 56: 1019-1032 (1989)). Preferred TAT transporter sequences for use in the JNK inhibitor of the present invention are preferably characterized by the presence of the TAT basic region amino acid sequence (amino acids 49-57 of naturally-occurring TAT protein); the absence of the TAT cysteine-rich region amino acid sequence (amino acids 22-36 of naturally-occurring TAT protein) and the absence of the TAT exon 2-encoded carboxy-terminal domain (amino acids 73-86 of naturally-occurring TAT protein). More preferably, the transporter sequence in the JNK inhibitor of the present invention may be selected from an amino acid sequence containing TAT residues 48-57 or 49 to 57 or variants thereof.

Preferably, the transporter sequence in a given JNK inhibitor of the present invention also exhibits D-amino acids, for example in order to improve stability towards proteases. Particularly preferred are transporter sequences which exhibit a specific order of alternating D- and L-amino acids. Such order of alternating D- and L-amino acids (the motif) may follow –without being limited thereto - the pattern of any one of SEQ ID NOs: 28-30:

$d_lLLL_xd_mLLL_yd_n$ (SEQ ID NO: 28);

$dLLLd(LLLd)_a$ (SEQ ID NO: 29); and/or

dLLLdLLLd (SEQ ID NO: 30);

wherein:	d	is a D-amino acid;
	L	is a L-amino acid;
	a	is 0 – 3, preferably 0-2, more preferably 0, 1, 2 or 3, even more preferably 0, 1, or 2 and most preferably 1;
	l, m and n	are independently from each other 1 or 2, preferably 1;
	x and y	are independently from each other 0, 1 or 2, preferably 1.

Said order of D- and L-amino acids (motif) becomes relevant when the transporter sequence is synthesized, i.e. while the amino acid sequence (i.e. the type of side chain residues) remains unaltered, the respective isomers alternate. For example, a known transporter sequence derived

from HIV TAT is RKKRRQRRR (SEQ ID NO: 43). Applying the D-/L amino acid order of SEQ ID NO: 30 thereto would yield rKKRrQRRr (SEQ ID NO: 46).

In a particular embodiment the transporter sequence of the JNK inhibitor of the present invention may comprise at least one sequence according to rXXXrXXXr (SEQ ID NO: 31), wherein:

r represents an D-enantiomeric arginine;

X is any L-amino acid (including glycine);

and wherein each X may be selected individually and independently of any other X within SEQ ID NO: 31. Preferably at least 4 out of said 6 X L-amino acids within SEQ ID NO: 31 are K or R. In another embodiment the JNK inhibitor according to the present invention comprises the transporter sequence $rX_1X_2X_3rX_4X_5X_6r$ (SEQ ID NO: 32), wherein X_1 is K, X_2 is K, X_3 is R and X_4, X_5, and X_6 are any L-amino acid (including glycine) selected independently from each other. Similarly, the transporter sequence of the JNK inhibitor according to the present invention may comprise the sequence $rX_1X_2X_3rX_4X_5X_6r$ (SEQ ID NO: 33), wherein X_4 is Q, X_5 is R, X_6 is R and X_1, X_2, and X_3 are any L-amino acid (including glycine) selected independently from each other. The inventive JNK inhibitor may also comprise the sequence $rX_1X_2X_3rX_4X_5X_6r$ (SEQ ID NO: 34), wherein one, two, three, four, five or six X amino acid residues are chosen from the group consisting of: X_1 is K, X_2 is K, X_3 is R, X_4 is Q, X_5 is R, X_6 is R, while the remaining X amino acid residues not selected from above group may be any L-amino acid (including glycine) and are selected independently from each other. X_1 is then preferably Y and/or X_4 is preferably K or R.

Examples of transporter sequences for use in the inventive JNK inhibitor molecule may be selected, without being limited thereto, from sequences as given in table 2 below, (SEQ ID NOs: 31-170) or from any fragment or variant or chemically modified derivative thereof (preferably it retains the function of translocating across a biological membrane).

Table 2:
Examples for transporter (poly-)peptide sequences for use in the JNK-inhibitors according to the present invention

SEQUENCE/PEPTIDE NAME	SEQ ID NO	AA	SEQUENCE
r3 (generic)	31	9	rXXXrXXXr
r3 (generic; right half)	32	9	$rKKRrX_4X_5X_6r$
r3 (generic; left half)	33	9	$rX_1X_2X_3rQRRr$
r3 (generic; individual)	34	9	$rX_1X_2X_3rX_4X_5X_6r$
TAT (1-86)	35	86	MEPVDPRLEP WKHPGSQPKT ACTNCYCKKC CFHCQVCFIT KALGISYGRK KRRQRRRPPQ GSQTHQVSLS KQPTSQSRGD PTGPKE
TAT (37-72)	36	36	CFITKALGIS YGRKKRRQRR RPPQGSQTHQ VSLSKQ

TAT (37-58)	37	22	CFITKALGIS YGRKKRRQRR RP
TAT (38-58)GGC	38	24	FITKALGISY GRKKRRQRRR PGGC
TAT CGG(47-58)	39	15	CGGYGRKKRR QRRRP
TAT (47-58)GGC	40	15	YGRKKRRQRR RPGGC
TAT (1-72) Mut Cys/Ala 72	41	56	MEPVDPRLEP WKHPGSQPKT AFITKALGIS YGRKKRRQRR RPPQGSQTHQ VSLSKQ
L-TAT (s1a)	42	10	GRKKRRQRRR (NH_2-GRKKRRQRRR-COOH)
L-TAT (s1b)	43	9	RKKRRQRRR (NH_2-GRKKRRQRRR-COOH)
L-TAT (s1c)	44	11	YDRKKRRQRRR
D-TAT	45	9	rrrqrrkkr
r_3-L-TAT	46	9	rKKRrQRRr
r_3-L-TATi	47	9	rRRQrRKKr
βA-r_3-L-TAT	48	9	βA-rKKRrQRRr (βA: beta alanine)
βA-r_3-L-TATi	49	9	βA-rRRQrRKKr (βA: beta alanine)
FITC-βA-r_3-L-TAT	50	9	FITC-βA-rKKRrQRRr (βA: beta alanine)
FITC-βA-r_3-L-TATi	51	9	FITC-βA-rRRQrRKKr (βA: beta alanine)
TAT(s2-1)	52	9	rAKRrQRRr
TAT(s2-2)	53	9	rKARrQRRr
TAT(s2-3)	54	9	rKKArQRRr
TAT(s2-4)	55	9	rKKRrARRr
TAT(s2-5)	56	9	rKKRrQARr
TAT(s2-6)	57	9	rKKRrQRAr
TAT(s2-7)	58	9	rDKRrQRRr
TAT(s2-8)	59	9	rKDRrQRRr
TAT(s2-9)	60	9	rKKDrQRRr
TAT(s2-10)	61	9	rKKRrDRRr
TAT(s2-11)	62	9	rKKRrQDRr
TAT(s2-12)	63	9	rKKRrQRDr
TAT(s2-13)	64	9	rEKRrQRRr
TAT(s2-14)	65	9	rKERrQRRr
TAT(s2-15)	66	9	rKKErQRRr
TAT(s2-16)	67	9	rKKRrERRr
TAT(s2-17)	68	9	rKKRrQERr
TAT(s2-18)	69	9	rKKRrQREr
TAT(s2-19)	70	9	rFKRrQRRr

TAT(s2-20)	71	9	rKFRrQRRr
TAT(s2-21)	72	9	rKKFrQRRr
TAT(s2-22)	73	9	rKKRrFRRr
TAT(s2-23)	74	9	rKKRrQFRr
TAT(s2-24)	75	9	rKKRrQRFr
TAT(s2-25)	76	9	rRKRrQRRr
TAT(s2-26)	77	9	rKRRrQRRr
TAT(s2-27)	78	9	rKKKrQRRr
TAT(s2-28)	79	9	rKKRrRRRr
TAT(s2-29)	80	9	rKKRrQKRr
TAT(s2-30)	81	9	rKKRrQRKr
TAT(s2-31)	82	9	rHKRrQRRr
TAT(s2-32)	83	9	rKHRrQRRr
TAT(s2-33)	84	9	rKKHrQRRr
TAT(s2-34)	85	9	rKKRrHRRr
TAT(s2-35)	86	9	rKKRrQHRr
TAT(s2-36)	87	9	rKKRrQRHr
TAT(s2-37)	88	9	rIKRrQRRr
TAT(s2-38)	89	9	rKIRrQRRr
TAT(s2-39)	90	9	rKKIrQRRr
TAT(s2-40)	91	9	rKKRrIRRr
TAT(s2-41)	92	9	rKKRrQIRr
TAT(s2-42)	93	9	rKKRrQRIr
TAT(s2-43)	94	9	rLKRrQRRr
TAT(s2-44)	95	9	rKLRrQRRr
TAT(s2-45)	96	9	rKKLrQRRr
TAT(s2-46)	97	9	rKKRrLRRr
TAT(s2-47)	98	9	rKKRrQLRr
TAT(s2-48)	99	9	rKKRrQRLr
TAT(s2-49)	100	9	rMKRrQRRr
TAT(s2-50)	101	9	rKMRrQRRr
TAT(s2-51)	102	9	rKKMrQRRr
TAT(s2-52)	103	9	rKKRrMRRr
TAT(s2-53)	104	9	rKKRrQMRr
TAT(s2-54)	105	9	rKKRrQRMr

TAT(s2-55)	106	9	rNKRrQRRr
TAT(s2-56)	107	9	rKNRrQRRr
TAT(s2-57)	108	9	rKKNrQRRr
TAT(s2-58)	109	9	rKKRrNRRr
TAT(s2-59)	110	9	rKKRrQNRr
TAT(s2-60)	111	9	rKKRrQRNr
TAT(s2-61)	112	9	rQKRrQRRr
TAT(s2-62)	113	9	rKQRrQRRr
TAT(s2-63)	114	9	rKKQrQRRr
TAT(s2-64)	115	9	rKKRrKRRr
TAT(s2-65)	116	9	rKKRrQQRr
TAT(s2-66)	117	9	rKKRrQRQr
TAT(s2-67)	118	9	rSKRrQRRr
TAT(s2-68)	119	9	rKSRrQRRr
TAT(s2-69)	120	9	rKKSrQRRr
TAT(s2-70)	121	9	rKKRrSRRr
TAT(s2-71)	122	9	rKKRrQSRr
TAT(s2-72)	123	9	rKKRrQRSr
TAT(s2-73)	124	9	rTKRrQRRr
TAT(s2-74)	125	9	rKTRrQRRr
TAT(s2-75)	126	9	rKKTrQRRr
TAT(s2-76)	127	9	rKKRrTRRr
TAT(s2-77)	128	9	rKKRrQTRr
TAT(s2-78)	129	9	rKKRrQRTr
TAT(s2-79)	130	9	rVKRrQRRr
TAT(s2-80)	131	9	rKVRrQRRr
TAT(s2-81)	132	9	rKKVrQRRr
TAT(s2-82)	133	9	rKKRrVRRr
TAT(s2-83)	134	9	rKKRrQVRr
TAT(s2-84)	135	9	rKKRrQRVr
TAT(s2-85)	136	9	rWKRrQRRr
TAT(s2-86)	137	9	rKWRrQRRr
TAT(s2-87)	138	9	rKKWrQRRr
TAT(s2-88)	139	9	rKKRrWRRr
TAT(s2-89)	140	9	rKKRrQWRr

TAT(s2-90)	141	9	rKKRrQRWr
TAT(s2-91)	142	9	rYKRrQRRr
TAT(s2-92)	143	9	rKYRrQRRr
TAT(s2-93)	144	9	rKKYrQRRr
TAT(s2-94)	145	9	rKKRrYRRr
TAT(s2-95)	146	9	rKKRrQYRr
TAT(s2-96)	147	9	rKKRrQRYr
TAT(s2-97)	148	8	rKKRrQRr
TAT(s2-98)	149	9	rKKRrQRrK
TAT(s2-99)	150	9	rKKRrQRrR
r_3R_6	151	9	rRRRrRRRr
L-R_9	152	9	RRRRRRRRR
L-R_8	153	8	RRRRRRRR
L-R_7	154	7	RRRRRRR
L-R_6	155	6	RRRRRR
L-R_5	156	5	RRRRR
r_9	157	9	rrrrrrrrr
r_5R_4 (D/L)	158	9	rRrRrRrRr
r_5R_4 (DD/LL)	159	9	rrRRrrRRr
PTD-4	160	11	YARAAARQARA
PTD-4 (variant 1)	161	11	WARAAARQARA
PTD-4 (variant 2)	162	11	WARAQRAAARA
L-P1 Penetratin	163	16	RQVKVWFQNRRMKWKK
D-P1 Penetratin	164	16	KKWKMRRNQFWVKVQR
JNKI, bestfit	165	17	WKRAAARKARAMSLNLF
JNKI, bestfit (variant 1)	166	17	WKRAAARAARAMSLNLF
MDCK transcytose sequence	167	9	RYRGDLGRR
YKGL	168	4	YKGL
P1	169	4	RRTK
P66	170	4	RRPK

As mentioned above, transporter sequences may also be selected from fragments or variants of the above sequences of table 2 (with the proviso that such fragment or variant retain preferably the function to provide for translocation across biological membranes). In this specific context, variants and/or fragments of those transporter sequences preferably comprise a peptide sequence

sharing at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 85%, preferably at least 90%, more preferably at least 95% and most preferably at least 99% sequence identity over the whole length of the sequence of such a transporter sequence as defined in Table 2. In this specific context, a "fragment" of a transporter sequence as defined in Table 2, is preferably to be understood as a truncated sequence thereof, i.e. an amino acid sequence, which is N-terminally, C-terminally and/or intrasequentially truncated compared to the amino acid sequence of the original sequence.

Furthermore, a "variant" of a transporter sequence or its fragment as defined above, is preferably to be understood as a sequence wherein the amino acid sequence of the variant differs from the original transporter sequence or a fragment thereof as defined herein in one or more mutation(s), such as one or more substituted, (or, if necessary, inserted and/or deleted) amino acid(s). Preferably, variants of such a transporter sequence as defined above have the same biological function or specific activity compared to the respective original sequence, i.e. provide for transport, e.g. into cells or the nucleus. In this context, a variant of such a transporter sequence as defined above may for example comprise about 1 to 50, 1 to 20, more preferably 1 to 10 and most preferably 1 to 5, 4, 3, 2 or 1 amino acid alterations. Variants of such a transporter sequence as defined above may preferably comprise conservative amino acid substitutions. The concept of conservative amino acid substitutions is known in the art and has already been set out above for the JNK inhibitory (poly-)peptide sequence and applies here accordingly.

The length of a transporter sequence incorporated in the JNK inhibitor of the present invention may vary. It is contemplated that in some embodiments the transporter sequence of the JNK inhibitor according to the present invention is less than 150, less than 140, less than 130, less than 120, less than 110, less than 100, less than 90, less than 80, less than 70, less than 60, less than 50, less than 40, less than 30, less than 20, and/or less than 10 amino acids in length.

Whether a specific transporter sequence is still functional in the context of the JNK inhibitor according to the present invention may easily be determined by a person skilled in the art. For instance, the JNK inhibitor comprising a transporter domain may be fused to a label, e.g. a fluorescent protein such as GFP, a radioactive label, an enzyme, a fluorophore, an epitope etc. which can be readily detected in a cell. Then, the JNK inhibitor comprising the transporter sequence and the label is transfected into a cell or added to a culture supernatant and permeation of cell membranes can be monitored by using biophysical and biochemical standard methods (for example flow cytometry, (immuno)fluorescence microscopy etc.).

Specific examples of JNK inhibitors according to the present invention comprising a transporter sequence are given in table 3:

Table 3:
Examples for JNK inhibitors comprising an inhibitory (poly-)peptide sequence and a transporter sequence

Amino acid sequence	AA	SEQ ID NO:
rKKRrQRRrRPkRPTTLNLf	20	171
rKKRrQRRrRPkRPaTLNLf	20	172
rKKRrQRRrRPkRPTTLrLf	20	173
rKKRrQRRrRPTTLNLf	17	174
rKKRrQRrRPTTLNLf	16	175
rKKRrQRRrRPkRPTTLNLw	20	176
rKKRrQRRrRPkRPTDLNLf	20	177
rKKRrQRRrRPTTLrLw	17	178
rKKRrQRrRPTTLrLw	16	179
rKKRrQRRrRPTDLrLw	17	180
rKKRrQRrRPTDLrLw	16	181
rKKRrQRRrRPaTLNLf	17	182
rKKRrQRrRPaTLNLf	16	183
rKKRrQRrKRPaTLNLf	17	184
rKKRrQRRrRPkRPsTLNLf	20	185
rKKRrQRRrRPkRPqTLNLf	20	186
rKKRrQRRrRPkRPkTLNLf	20	187
rKKRrQRRrGKRKALKLf	18	188
rKKRrQRRrGKRKALrLf	18	189
rKKRrQRRrRKALrLf	16	190

As mentioned above, in a particular embodiment of the present invention the transporter sequence and the inhibitory (poly-)peptide sequence may overlap. In other words, the N-terminus of the transporter sequence may overlap with the C-terminus of the inhibitory (poly-)peptide sequence or the C-terminus of the transporter sequence may overlap with the N-terminus of the inhibitory (poly-)peptide sequence. The latter embodiment is particularly preferred. Preferably, the transporter sequence overlaps by one, two or three amino acid residues with the inhibitory (poly-)peptide sequence. In such scenario a given transporter sequence may overlap with SEQ ID NO:1

or the respective variants thereof at position 1 (X1), position 1 and 2 (X1, X2), positions 1, 2 and 3 (X1, X2, X3).

SEQ ID NOs: 174, 175, 178, 179, 180, 181, 182, 183, 184, 188, 189 and 190 are good examples for JNK inhibitors according to the present invention, wherein transporter sequence and the inhibitory (poly-)peptide sequence overlap, e.g. rKKRrQ*RRrRPTTLNLf* (SEQ ID NO: 174) is an overlap of SEQ ID NO: 46 (underlined) and SEQ ID NO: 11 (italics).

Certainly the JNK inhibitor according to the present invention may also be selected from JNK inhibitors, which are a variant of any one of the JNK inhibitors according to SEQ ID NOs: 171-190. Preferably, such variant shares at least 50%, more preferably at least 55%, more preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, most preferably at least 95% sequence identity with the sequence of SEQ ID NOs: 171-190, in particular with SEQ ID NO: 172,

with the proviso that with respect to the inhibitory (poly-)peptide sequence within said sequences of SEQ ID NOs: 171-190 (see for reference inhibitory (poly-)peptide sequence of SEQ ID NO: 1 and specific examples of SEQ ID NOs: 2-27)) such sequence sharing sequence identity

a) maintains the L-arginine (R) residue on position 4 within the inhibitory (poly-)peptide sequence,

b) maintains the two L-leucine (L) residues at position 8 and 10 (positions 7 and 9 with regard to SEQ ID NOs: 25-27) within the inhibitory (poly-)peptide sequence,

c) exhibits at least one, at least two, at least three, at least four, at least five or six D-amino acid(s) at the respective positions corresponding to the amino acids selected from the group consisting of X1, X2, X3, X5, X7 and or X8 of SEQ ID NO: 1 and respective positions in SEQ ID NOs: 2-27, more preferably exhibits at least one, at least two, at least three or four D-amino acid(s) at the positions corresponding to the amino acids selected from the group consisting of X3, X5, X7 and X8 of SEQ ID NO: 1 and respective positions in SEQ ID NOs: 2-27, and

d) still inhibits JNK activity (i.e. is a JNK inhibitor as defined herein).

In view of said definition and for sake of clarity the residues which may not be changed in variants of JNK inhibitors comprising SEQ ID NOs: 171-190 (see a) and b) in the above definition) are underlined in table 3.

The non-identical amino acids in the variants of JNK inhibitors comprising SEQ ID NOs: 171-190 are preferably the result of conservative amino acid substitutions (see above). Certainly, the further possible substitutions mentioned above are also contemplated for variants of JNK inhibitors comprising SEQ ID NOs: 171-190. Likewise, the present invention certainly also contemplates variants of any one of the JNK inhibitors according to SEQ ID NOs: 171-190, which deviate from the original sequence not or not exclusively in the inhibitory (poly-)peptide sequence, but exhibits variant residues in the transporter sequence. For variants and fragments of transporter sequences see in particular respective disclosure above.

As mentioned previously, the transporter sequence and the JNK inhibitory (poly)-peptide sequence of the JNK inhibitors according to the present invention need not necessarily be directly joined to each other. They may also be spaced apart, e.g. by intermediate (poly-)peptide sequences. Preferred intermediate sequences separating the inhibitory (poly-)peptide sequences and other (functional) sequences such as transporter sequences consist of short peptide sequences less than 10 amino acids in length like a hexaamer, a pentamer, a tetramer, a tripeptide or even only a dipeptide or a single amino acid residue. Particularly preferred intermediate sequence are one, two or more copies of di-proline, di-glycine, di-arginine and/or di-lysine, all either in L-amino acid form only, or in D-amino acid form only, or with mixed D- and L-amino acids. Certainly, other known peptide spacer sequences may be employed as well.

A particularly preferred JNK inhibitor according to the present invention comprises SEQ ID NO: 8 (or a sequence sharing sequence identity with SEQ ID NO: 8 with the scope and limitations defined further above) and a transporter sequence. The transporter sequence is preferably selected from any one of SEQ ID Nos: 31-170 or variants thereof as defined herein, even more preferably from any one of SEQ ID NOs: 31-34 and 46-151. A particularly preferred embodiment of a JNK inhibitor according to the present invention is a JNK inhibitor comprising SEQ ID NO: 8 and SEQ ID NO: 46 (or sequences sharing respective sequence identity thereto within the scope and limitations defined further above). A preferred example is a JNK inhibitor comprising the sequence of SEQ ID NO: 172 or respective variants thereof varying in the transporter sequence and/or the inhibitory (poly-)peptide sequence as defined herein.

In a further aspect the present invention relates to a JNK inhibitor comprising

a) an inhibitory (poly-)peptide comprising a sequence from the group of sequences consisting of RPTTLNLF (SEQ ID NO: 191), KRPTTLNLF (SEQ ID NO: 192), RRPTTLNLF and/or RPKRPTTLNLF (SEQ ID NO: 193), and

b) a transporter sequence, preferably a transporter sequence selected from the transporter sequences disclosed in table 2 or variants/fragments thereof, even more preferably selected from SEQ ID NOs: 31-34 and 46-151 or respective variants or fragments thereof.

The transporter sequence and the inhibitory (poly-)peptide sequence may overlap. Preferred transporter sequences for said embodiment of the invention are particularly the transporter sequence of SEQ ID NO: 46, preferably joined (e.g. directly) to the N-Terminus of the inhibitory (poly-)peptide sequence.

A JNK inhibitor of the present invention may also be a JNK inhibitor comprising or consisting of the sequence GRKKRRQRRRPPKRPTTLNLFPQVPRSQD (SEQ ID NO: 194), or the sequence GRKKRRQRRRPTTLNLFPQVPRSQD (SEQ ID NO: 195).

In a further aspect the present invention relates to a (poly-)peptide comprising a transporter sequence selected from the group of sequences consisting of rKKRrQRr (SEQ ID NO: 148), rKKRrQRrK (SEQ ID NO: 149), and/or rKKRrQRrR (SEQ ID NO: 150).

As used herein, comprising a certain sequence or a certain SEQ ID NO: usually implies that (at least) one copy of said sequence is present, e g. in the JNK inhibitor molecule. For example, one inhibitory (poly-)peptide sequence will usually suffice to achieve sufficient inhibition of JNK activity. However, the inventor certainly contemplate that the use of two or more copies of the respective sequence (e.g. two or more copies of an inhibitory (poly-)peptide sequence of different or same type and/or two or more copies of a transporter sequence of different or the same type) may also employed as long as the overall ability of the resulting molecule to inhibit JNK activity is not abolished (i.e. the respective molecule is still a JNK inhibitor as defined herein).

The inventive JNK inhibitors may be obtained or produced by methods well-known in the art, e.g. by chemical synthesis via solid-phase peptide synthesis using Fmoc (9-fluorenylmethyloxycarbonyl) strategy, i.e. by successive rounds of Fmoc deprotection and Fmoc-amino acid coupling cycles. A commercial service offering such peptide synthesis is provided by many companies, for example the company PolyPeptide (Straßbourg, France).

The JNK inhibitors for use according to the present invention may optionally be further modified, in particular at the amino acid residues of the inhibitory (poly-peptide) sequence. Possible modifications may for example be selected from the group consisting of:

(i) radioactive labels, i.e. radioactive phosphorylation or a radioactive label with sulphur, hydrogen, carbon, nitrogen, etc.;
(ii) colored dyes (e.g. digoxygenin, etc.);
(iii) fluorescent groups (e.g. fluorescein, etc.);
(iv) chemoluminescent groups;
(v) groups for immobilization on a solid phase (e.g. His-tag, biotin, strep-tag, flag-tag, antibodies, epitopes, etc.);
(vi) pegylation,
(vii) glycosylation,
(viii) hesylation,
(ix) protease cleavage sites (e.g. for controlled release of the JNK inhibitor)
(x) peptide backbone modifications (e.g. (ΨCH_2-NH) bonds)
(xi) protection of amino acid side chain residues,
(xii) protection of N- and/or C-terminus (e.g. N-terminal amidation or C-terminal acetylation)
(xiii) a combination of elements of two or more of the elements mentioned under (i) to (xii).

Particularly preferred are modifications selected from (i) to (xi) and combinations of elements of two or more of the elements mentioned under (i) to (xi). In this context the present invention relates in a further aspect to a JNK inhibitor as disclosed herein modified with modifications selected from (i) to (xi) or modified with a combination of two or more of the elements mentioned under (i) to (xi), and a pharmaceutical composition (see below) comprising such modified JNK inhibitor.

Pharmaceutical compositions

The JNK inhibitors as defined according to the invention can be formulated in a pharmaceutical composition, which may be applied in the prevention or treatment of any of the diseases as defined herein. Typically, such a pharmaceutical composition used according to the present invention includes as an active component a JNK inhibitor as defined herein, in particular a JNK inhibitor comprising or consisting of an inhibitory (poly-)peptide sequence according to SEQ ID NO: 1, as defined herein. Preferably, the active compound is a JNK inhibitor comprising or consisting of an inhibitory (poly-)peptide sequence according to any one of SEQ ID NOs: 2-27; or, if a transporter sequence is attached, according to any one of SEQ ID NOs: 171-190.

The inventors of the present invention additionally found, that the JNK-inhibitors as defined herein, in particular if fused to a transporter sequence; exhibit a particular well uptake rate into cells involved in the diseases of the present invention. Therefore, the amount of a JNK-inhibitor inhibitor in the pharmaceutical composition to be administered to a subject, may –without being limited thereto - have a very low dose. Thus, the dose may be much lower than for peptide drugs known in the art, such as DTS-108 (Florence Meyer-Losic et al., Clin Cancer Res., 2008, 2145-53). This has several positive aspects, for example a reduction of potential side reactions and a reduction in costs.

Preferably, the dose (per kg bodyweight) is in the range of up to about 10 mmol/kg, preferably up to about 1 mmol/kg, more preferably up to about 100 µmol/kg, even more preferably up to about 10 µmol/kg, even more preferably up to about 1 µmol/kg, even more preferably up to about 100 nmol/kg, most preferably up to about 50 nmol/kg.

Thus, the dose range may preferably be from about 1 pmol/kg to about 1 mmol/kg, from about 10 pmol/kg to about 0,1 mmol/kg, from about 10 pmol/kg to about 0,01 mmol/kg, from about 50 pmol/kg to about 1 µmol/kg, from about 100 pmol/kg to about 500 nmol/kg, from about 200 pmol/kg to about 300 nmol/kg, from about 300 pmol/kg to about 100 nmol/kg, from about 500 pmol/kg to about 50 nmol/kg, from about 750 pmol/kg to about 30 nmol/kg, from about 250 pmol/kg to about 5 nmol/kg, from about 1 nmol/kg to about 10 nmol/kg, or a combination of any two of said values.

In this context, prescription of treatment, e.g. decisions on dosage etc. when using the above pharmaceutical composition is typically within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in REMINGTON'S PHARMACEUTICAL SCIENCES, 16th edition, Osol, A. (ed), 1980. Accordingly, a "safe and effective amount" for components of the pharmaceutical compositions as used according to the present invention means an amount of each or all of these components, that is sufficient to significantly induce a positive modification of diseases or disorders strongly related to JNK signalling as defined herein. At the same time, however, a "safe and effective amount" is small enough to avoid serious side-effects, that is to say to permit a sensible relationship between advantage and risk. The determination of these limits typically lies within the scope of sensible medical judgment. A "safe and effective amount" of such a component will vary in connection with the particular condition to be treated and also with the age and physical condition of the

patient to be treated, the severity of the condition, the duration of the treatment, the nature of the accompanying therapy, of the particular pharmaceutically acceptable carrier used, and similar factors, within the knowledge and experience of the accompanying doctor. The pharmaceutical compositions according to the invention can be used according to the invention for human and also for veterinary medical purposes.

The pharmaceutical composition as used according to the present invention may furthermore comprise, in addition to one or more of the JNK inhibitors, a (compatible) pharmaceutically acceptable carrier, excipient, buffer, stabilizer or other materials well known to those skilled in the art.

In this context, the expression "(compatible) pharmaceutically acceptable carrier" preferably includes the liquid or non-liquid basis of the composition. The term "compatible" means that the constituents of the pharmaceutical composition as used herein are capable of being mixed with the pharmaceutically active component as defined above and with one another component in such a manner that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the composition under usual use conditions. Pharmaceutically acceptable carriers must, of course, have sufficiently high purity and sufficiently low toxicity to make them suitable for administration to a person to be treated.

If the pharmaceutical composition as used herein is provided in liquid form, the pharmaceutically acceptable carrier will typically comprise one or more (compatible) pharmaceutically acceptable liquid carriers. The composition may comprise as (compatible) pharmaceutically acceptable liquid carriers e.g. pyrogen-free water; isotonic saline or buffered (aqueous) solutions, e.g. phosphate, citrate etc. buffered solutions, vegetable oils, such as, for example, groundnut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil from theobroma; polyols, such as, for example, polypropylene glycol, glycerol, sorbitol, mannitol and polyethylene glycol; alginic acid, etc. Particularly for injection of the pharmaceutical composition as used herein, a buffer, preferably an aqueous buffer, may be used.

If the pharmaceutical composition as used herein is provided in solid form, the pharmaceutically acceptable carrier will typically comprise one or more (compatible) pharmaceutically acceptable solid carriers. The composition may comprise as (compatible) pharmaceutically acceptable solid carriers e.g. one or more compatible solid or liquid fillers or diluents or encapsulating compounds may be used as well, which are suitable for administration to a person. Some examples of such (compatible) pharmaceutically acceptable solid carriers are e.g. sugars, such as, for example,

lactose, glucose and sucrose; starches, such as, for example, corn starch or potato starch; cellulose and its derivatives, such as, for example, sodium carboxymethylcellulose, ethylcellulose, cellulose acetate; powdered tragacanth; malt; gelatin; tallow; solid glidants, such as, for example, stearic acid, magnesium stearate; calcium sulphate, etc.

The precise nature of the (compatible) pharmaceutically acceptable carrier or other material may depend on the route of administration. The choice of a (compatible) pharmaceutically acceptable carrier may thus be determined in principle by the manner in which the pharmaceutical composition as used according to the invention is administered. The pharmaceutical composition as used according to the invention can be administered, for example, systemically. Routes for administration include, for example, parenteral routes (e.g. via injection), such as intravenous, intramuscular, subcutaneous, intradermal, or transdermal routes, etc., enteral routes, such as oral, or rectal routes, etc., topical routes, such as nasal, or intranasal routes, etc., or other routes, such as epidermal routes or patch delivery. Also contemplated (in particular for eye related diseases) are instillation, intravitreal, and subconjunctival administration. Likewise administration may occur intratympanical, for example if ear related diseases are treated.

The suitable amount of the pharmaceutical composition to be used can be determined by routine experiments with animal models. Such models include, without implying any limitation, rabbit, sheep, mouse, rat, dog and non-human primate models. Preferred unit dose forms for injection include sterile solutions of water, physiological saline or mixtures thereof. The pH of such solutions should be adjusted to about 7.4. Suitable carriers for injection include hydrogels, devices for controlled or delayed release, polylactic acid and collagen matrices. Suitable pharmaceutically acceptable carriers for topical application include those, which are suitable for use in lotions, creams, gels and the like. If the compound is to be administered per orally, tablets, capsules and the like are the preferred unit dose form. The pharmaceutically acceptable carriers for the preparation of unit dose forms, which can be used for oral administration are well known in the prior art. The choice thereof will depend on secondary considerations such as taste, costs and storability, which are not critical for the purposes of the present invention, and can be made without difficulty by a person skilled in the art.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier as defined above, such as gelatin, and optionally an adjuvant. Liquid pharmaceutical compositions for oral administration generally may include a liquid carrier as defined above, such as water, petroleum, animal or vegetable oils, mineral oil or

synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be included, as required. Whether it is a polypeptide, peptide, or nucleic acid molecule, other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is preferably in a "prophylactically effective amount or a "therapeutically effective amount" (as the case may be), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated.

Treatment of a disease as defined herein typically includes administration of a pharmaceutical composition as defined above. The JNK inhibitors of the present invention will modulate the JNK activity in the subject. The term "modulate" includes in particular the suppression of phosphorylation of c-jun, ATF2 or NFAT4 in any of the above diseases, for example, by using at least one JNK inhibitor comprising or consisting of an inhibitory (poly)peptide sequence according to any of sequences of SEQ ID NOs: 2 to 27, potentially comprising an additional transporter sequence, as a competitive inhibitor of the natural c-jun, ATF2 and NFAT4 binding site in a cell. The term "modulate" also includes suppression of hetero- and homomeric complexes of transcription factors made up of, without being limited thereto, c-jun, ATF2, or NFAT4 and their related partners, such as for example the AP-1 complex that is made up of c-jun, AFT2 and c-fos.

Treatment of a subject with the pharmaceutical composition as disclosed above may be typically accomplished by administering (*in vivo*) an ("therapeutically effective") amount of said pharmaceutical composition to a subject, wherein the subject may be e.g. a human subject or an animal. The animal is preferably a non-human mammal, e.g., a non-human primate, mouse, rat, dog, cat, cow, horse or pig. The term "therapeutically effective" means that the active component of the pharmaceutical composition is of sufficient quantity to ameliorate the diseases and disorders as discussed herein.

Diseases and disorders

The gist of the present invention is to use the above disclosed JNK inhibitors and pharmaceutical compositions in a method for treatment of the human or animal body by therapy, in particular of the human body. As mentioned above JNK signalling is involved in a multitude of diverse disease states and disorder and inhibition of said signalling has proposed and successfully tested for many of these. The inventors of the present invention found that the JNK inhibitors disclosed herein are effective JNK inhibitors and are thus equally suitable for the treatment of the diseases as disclosed in the art.

Treatment of a human or animal body by therapy, as used herein, refers to any kind of therapeutic treatment of a respective subject. It includes for example prevention of onset of the disease or symptoms (prophylaxis), i.e. typically prior to manifestation of the disease in the patient. The term also includes the "mere" treatment of symptoms of a given disease, i.e. the treatment will ameliorate pathogenesis by reducing disease associated symptoms, without necessarily curing the underlying cause of the disease and symptoms. Certainly, curing the underlying cause of the disease is also encompassed by the term. The term also encompasses a treatment which delays or even stops progression of the respective disease.

In one embodiment the JNK inhibitors according to the present invention may be administered for example prophylactically prior to potential onset of a foreseeable disorder, e.g. prior to a planned surgical intervention or planned exposure to stressful stimuli. A surgical intervention could for example bear the risk of inflammation of the respective wound or neighbouring tissue (e.g. dry eye syndrome after surgical eye treatment, peri-implantitis after dental implantation treatment, rejection of the graft after transplantation, etc.). Exposure to stressful stimuli like radiation could lead to apoptosis of affected tissue and cells. In such scenario the JNK inhibitors according to the present invention may for example be administered at least once up to about 4 weeks in advance. The JNK inhibitors may for example be administered at least 24 hours, at least 48 hours, at least 1 week, at least 2 weeks or 4 weeks in advance.

The diseases and disorders to be treated with the JNK inhibitors as disclosed herein may be acute or chronic.

Due to the involvement of JNK signalling in a vast diversity of pathological conditions, the JNK inhibitors of the present invention may for example be used for the treatment of diseases of various organs, such as diseases of the eye, diseases of the bone, neural diseases, neuronal diseases,

neurodegenerative diseases, diseases of the skin, immune and/or autoimmune diseases, diseases of the eye, diseases of the mouth, inflammatory diseases, metabolic diseases, cardiovascular diseases, proliferative diseases (in particular cancers and tumors), diseases of the ear, diseases of the intestine, diseases of the respiratory system (e.g. lung diseases), infectious diseases, and various other diseases.

The JNK inhibitors of the present invention may be used for example for the treatment of inflammatory diseases including for example acute inflammation as well as chronic inflammation. The JNK inhibitors of the present invention may be used to treat any type of tissue inflammation, e.g. inflammation in the eye, inflammation in the mouth, inflammation of the respiratory system including in particular the lung, inflammation of the skin, inflammation within the cardiovascular system, inflammation of the brain, inflammation in the ear, etc. Some non-limiting examples for such inflammatory disease states are mucositis, stomatitis, peri-implantitis, retinitis, chorioiditis, keratoconjunctivitis sicca, inflammatory bowel diseases (IBD), uveitis (e.g. anterior uveitis, intermediate uveitis, posterior uveitis), periodontitis, COPD, asthma, pulpitis, rheumatoid arthritis, osteoarthritis, Crohn's disease, psoriatic arthritis, vasculitis, interstitial cystitis; acute inflammation at a site of infection or wound, meningitis, encephalitis, pneumonia, pharyngitis, tonsillitis, otitis (including otitis media), vasculitis, synovitis, enteritis, Crohn's disease, ulcerative colitis, graft rejection etc.

The JNK inhibitors as disclosed herein may for example be used in methods of treatment of ear diseases (in particular diseases of the inner ear), hearing loss (in particular acute hearing loss), damaged hair cell stereocilia, hair cell apoptosis, noise trauma, otitis, otitis media etc. Hearing loss and associated hair cell apoptosis are non-limiting examples for disorders resulting from stress situations for cells in which JNK inhibition can modulate the stress response and for example block apoptosis.

The JNK inhibitors of the present invention may also be used for the treatment of metabolic disorders, for example for the treatment of diabetes (type 1 or type 2, in particular type 1), Fabry disease, Gaucher disease, hypothermia, hyperthermia hypoxia, lipid histiocytosis, lipidoses, metachromatic leukodystrophy, mucopolysaccharidosis, Niemann Pick disease, obesity, and Wolman's disease. Hypothermia, hyperthermia and hypoxia are again non-limiting examples for stress situations for cells in which JNK inhibition can modulate the stress response and for example block apoptosis.

Likewise, the JNK inhibitors of the present invention may be used for the treatment of neural, neuronal and/or neurodegenerative diseases, respectively. Examples for such diseases are for example Alexander disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), apoplexy, Ataxia Telangiectasia, cut or otherwise disrupted axons, axotomy, brain lesions, CMT (Charcot-Marie-Tooth), corticobasal degeneration, dementia, diseases or disorders of the nervous system, dystonia, epilepsy, Farber's disease, Friedreich ataxia (SCA), gangliosidoses, Guillain-Barré syndrome, hereditary spastic paraplegia, Hirschsprung's disease, human immunodeficiency virus dementia, Huntington's disease, infarct of the brain, ischemic stroke, Krabbe disease, Lennox Gastaut Syndrome, lissencephaly, multiple sclerosis, myelodysplastic syndromes, myelopathy, AIDS-related neurodegenerative diseases, neurofibromatosis type 2 (NF-2), neurolatyerism, neuronal apoptosis, neuronal death, neuropathic pain, neuropathy, chemotherapy induced neuropathy, diabetes induced neuropathy, NMDA-induced neurotoxicity, pain, Parkinson's disease, parkinsonism, Pick's Disease, polyneuropathy, progressive supranuclear palsy, Sandhoff disease, spina bifida, stroke, Tay Sachs, TBI (diffuse axonal injury), treatment of dark neurone induced for example by an inflammatory pain, West Syndrome, spinal muscular atrophy etc.

With respect to autoimmune disorders, the JNK inhibitor peptides of the present invention may for example be used in a method of treatment of autoimmune diseases of the CNS, auto-inflammatory diseases, Celiac disease; Sjogren's syndrome, systemic lupus erythematosus etc.

Examples for bone diseases which may be treated with the JNK inhibitors of the present invention are for example arthritis, disc herniation, fibrodysplasia ossificans progressiva (FOP), osteoarthritis, osteopetrosis, osteoporosis, in particular diabetes induced osteoporosis, Paget's Disease, rheumatoid arthritis, etc.

Examples for skin diseases which may be treated with the JNK inhibitors of the present invention are for example psoriasis and lupus erythematosus.

Diseases of the eye, which may be treated with the JNK inhibitors of the present invention involve for example age-related macular degeneration (AMD); angioid streaks; anterior ischemic optic neuropathy; anterior uveitis; cataract, in particular age related cataract; central exudative chorioretinopathy; central serous chorioretinopathy; chalazion; chorioderemia; chorioiditis; choroidal sclerosis; conjunctivitis; cyclitis; diabetic retinopathy; dry eye syndrome; endophthalmitis; episcleritis; eye infection; fundus albipunctatus; gyrate atrophy of choroid and retina; hordeolum; inflammatory diseases of the blephara; inflammatory diseases of the choroid; inflammatory diseases of the ciliary body; inflammatory diseases of the conjunctiva; inflammatory

diseases of the cornea; inflammatory diseases of the iris; inflammatory diseases of the lacrimal gland; inflammatory diseases of the orbital bone; inflammatory diseases of the sclera; inflammatory diseases of the vitreous body; inflammatory diseases of the uvea; inflammatory diseases of the retina; intermediate uveitis; irititis; keratitis; Leber's disease; multifocal choroiditis; myositis of the eye muscle; neovascular maculopathy (e.g. caused by high myopia, tilted disc syndrome, choroidal osteoma or the like); NMDA induced retinotoxicity; non-chronic or chronic inflammatory eye diseases; Oguchi's disease; optic nerve disease; orbital phlegmon; panophtalmitis; panuveitis; post caspule opacification; posterior capsule opacification (PCO) (a cataract after-surgery complication); posterior uveitis; proliferative vitreoretinopathy; retinal artery occlusion; retinal detachment, retinal diseases; retinal injuries; retinal macroaneurysm; retinal pigment epithelium detachment; retinal vein occlusion; retinitis; retinitis pigmentosa; retinitis punctata albescens; retinopathy, in particular retinopathy of prematurity and diabetic retinopathy; scleritis; Stargardt's disease; treatment of inflamed ocular wounds and/or ocular wound edges; treatment of intraocular inflammation after eye surgery or trauma; uveitis; vitelliform macular dystrophy; etc.

Exemplary diseases of the mouth which may be treated with the JNK inhibitors as disclosed herein are periodontitis, in particular chronic periodontitis; mucositis, oral desquamative disorders, oral liquen planus, pemphigus vulgaris, pulpitis; stomatitis; temporomandibular joint disorder, peri-implantitis etc.

Likewise the JNK inhibitors of the present invention may - as already previously proposed for other JNK inhibitors - be used for the treatment of proliferative diseases like cancer and tumor diseases, such as acusticus neurinoma lung carcinomas; acute lymphocytic leukemia (L1, L2, L3); acute lymphoid leukaemia (ALL); acute myelogenous leukemia (AML); adenocarcinomas; anal carcinoma; bronchial carcinoma; cervix carcinoma; cervical cancer; astrocytoma; basalioma; cancer with Bcr-Abl transformation; bladder cancer; blastomas; bone cancer; brain metastases; brain tumours; breast cancer; Burkitt's lymphoma; carcinoids; cervical cancer; chronic lymphocytic leukaemia (CLL); chronic myeloid leukaemia (CML); colon cancer; colon carcinoma; corpus carcinoma; craniopharyngeomas; CUP syndrome; virus-induced tumours; EBV-induced B cell lymphoma; endometrium carcinoma; erytholeukemia (M6); esophagus cancer; gallbladder cancer; gastrointestinal cancer; gastrointestinal stromal tumors; gastrointestinal tumours; genitourinary cancer; glaucoma; glioblastoma; gliomas; head/neck tumours; hepatitis B-induced tumours; hepatocell carcinomas; hepatomas; herpes virus-induced tumours; Hodgkin's syndrome; HTLV-1-induced lymphomas; HTLV-2-induced lymphomas; insulinomas; intestinal cancer; Kaposi's sarcoma; kidney cancer; kidney carcinomas; laryngeal cancer; leukemia; lid tumour; liver

cancer; liver metastases; lung cancer; lymphoid cancer; lymphomas; malignant melanomas; mammary carcinomas; mantle cell lymphoma; medulloblastoma; megakaryoblastic leukemia (M7); melanoma, in particular malignant melanoma; meningioma; mesothelioma; monocytic leukemia (M5); multiple myeloma; mycosis fungoides; myeloblastic leukemia (M1); myeloblastic leukemia (M2); myelomonocytic leukemia (M4); neurinoma; non-Hodgkin's lymphomas; non-small cell carcinoma; non-small cell carcinoma of the lung; oesophageal cancer; oesophageal carcinoma; oligodendroglioma; ovarian cancer; ovarian carcinoma; pancreatic cancer; pancreatic carcinoma; papilloma virus-induced carcinomas; penis cancer; pituitary tumour; plasmocytoma; promyelocytic leukemia (M3); prostate cancer; prostate tumours; rectal tumours; rectum carcinoma; renal-cell carcinoma; retinoblastoma; sarcomas; Schneeberger's disease; small cell lung carcinomas; small intestine cancer; small intestine tumours; soft tissue tumours; spinalioma; squamous cell carcinoma; stomach cancer; testicular cancer; throat cancer; thymoma; thyroid cancer; thyroid carcinoma; tongue cancer; undifferentiated AML (M0); urethral cancer; uterine cancer; vaginal cancer; Von Hippel Lindau disease; vulval cancer; Wilms' Tumor; Xeroderma pigmentosum; etc.

Since JNK signalling is also involved in many cardiovascular diseases and disorders, the use of JNK inhibitors in the treatment of such diseases has already been suggested in the past. The inhibitors of the present invention may be used accordingly, e.g. for the treatment of cardiovascular diseases such as arterial hypertension; arteriosclerosis; arteriosclerotic lesions; Behcet's syndrome; bifurcations of blood vessels; cardiac hypertrophy; cardiavascular hypertrophy; cardiomyopathies, in particular chemotherapy induced cardiomyopathies; cerebral ischemia; coronary heart diseases; dilatation of the abdominal aorta; focal cerebral ischemia; global cerebral ischemia; heart hypertrophy; infrarenal aneurism hypertension; ischemia; myocardial infarct, in particular acute myocardial infarction; myocarditis; reperfusion; restenosis; vasculitis; Wegener's granulomatosis; etc.

The JNK inhibitors of the present invention may in the context of cardiovascular diseases also be used complementary to coronary artery bypass graft surgery (CABG surgery); percutaneous transluminal coronary angioplasty (PTCA); and/or stent treatment, for example to prevent or treat intimal hyperplasia resulting from said (surgical) treatment.

Diseases of the respiratory system and in particular lung diseases which may be treated effectively with the JNK inhibitors of the present invention are for example acute respiratory distress syndrome (ARDS); asthma; chronic illnesses involving the respiratory system; chronic obstructive

pulmonary disease (COPD); cystic fibrosis; inflammatory lung diseases; pneumonia; pulmonary fibrosis; etc.

Like the inhibitors in the prior art the inhibitors of the present invention may also be used to treat disease of the intestinal tract, e.g. colitis (e.g. atypical colitis, chemical colitis; collagenous colitis, distal colitis, diversion colitis; fulminant colitis, indeterminate colitis, infectious colitis, ischemic colitis, lymphocytic colitis, or microscopic colitis), Crohn's disease, gastroenteritis, Hirschsprung's disease, inflammatory digestive diseases; inflammatory bowel disease (IBD), Morbus Crohn, non-chronic or chronic digestive diseases, non-chronic or chronic inflammatory digestive diseases; regional enteritis; ulcerative colitis etc.

The JNK inhibitors of the present invention may also serve as therapeutic agent for the treatment of infectious diseases resulting from e.g. bacterial or viral infection. The JNK inhibitors as disclosed herein may for example prevent or ameliorate inflammatory reactions caused by said infections. Examples for such diseases states, which are not considered to be limiting, are viral encephalitis; viral induced cancers (e.g. as mentioned above), human immunodeficiency virus dementia, meningitis, meningoencephalitis, encephalomyelitis, tonsillitis, etc.

There are many other diseases, disease states and disorders for which the JNK inhibitors of the present invention can be used as treatment, for example Aarskog syndrome, acetaminophen hepatotoxicity; Alder-Reilly anomaly; alopecia areata; alpha-1-antitrypsin deficiency; anaphylaxis; apoptosis; apoptotic cell death; atypical hemolytic uremic syndrome; basopenia; basophilia; bipolar disorders; burns; cellular shear stress; Chedial-Higashi syndrome; DNA damage due to chemotherapeutic drugs; cholestasis; chromosome 11, Partial Monosomy 11q; chromosome 22, Trisomy Mosaic; chronic granulomatous disease; hepatitis, such as chronic or fulminant hepatitis; clinical depression; common variable hypogammaglobulinemia; congenital C3 deficiency; CTL protection from activation-induced cell death (AICD); deafness; depression and depressive disorders (in particular prevention of depressive disorders develop on a background of cytokine-induced sickness behaviour), DiGeorge's syndrome; diseases caused by defective apoptosis; diseases of the liver; diseases of the spine; diseases of the uterus; diseases states and symptoms due to exposure to DNA damaging agents and/or ionizing radiation and resulting cellular stress; Down Syndrome; Duchenne muscular dystrophy; ectodermal dysplasias; endometriosis; eosinopenia; eosinophilia; exocitoxic cell death; fetal alcohol syndrome; fibrosis; fibrotic disease; formation of fibrous tissue; free radicals (leading to cellular stress); graft rejection; Graft versus host Disease; hair loss; hemolytic uremic syndrome; hepatotoxicity; hyperalgesia, such as diabetes induced hyperalgesia; hyperthermia; hypoglycemia; hypothyroidism; idiopathic hypereosinophilic

syndrome; IgA nephropathy; infantile sex-linked agammaglobulinemia; inflammatory pain; infrarenal aneyrism; islet regeneration; islet transplantation; Job's syndrome (hyper-IgE); lazy leukocyte syndrome; leukocyte glucose-6-phosphate dehydrogenase deficiency; leukodystrophy; leukopenia; lymphocytic leukocytosis; lymphocytopenia; lymphocytosis; major depression; mania; maniac depression; Marfan syndrome; mastocytosis; May Hegglin Anomaly; membranoproliferative glomerulonephritis Type II; monocytopenia; monocytosis; myeloperoxidase deficiency-benign; myopathies; neutropenia; neutrophilia; Nezelof's syndrome; organ transplantation; oxidative stress injuries; Pelger-Huet anomaly; polycystic kidney diseases; post-dialysis syndrome; radiation syndromes; radiotherapy; renal diseases; renal failure; rescuing CTL from activation induced cell death; severe combined immunodeficiency disease; transplant rejection; transplantation; trisomy; unipolar depression; UV-induced injuries; Wiskott Aldrich syndrome; wound healing; etc.

The inventors of the present invention consider temporomandibular joint disorder, mucositis, stomatitis, oral liquen planus (desquamative disorder), Pemphigus vulgaris (desquamative disorder), periodontitis, chronic periodontitis, pulpitis, peri-implantitis, uveitis (anterior uveitis, intermediate uveitis, posterior uveitis), keratoconjunctivitis sicca (dry eye syndrome), coronary artery bypass graft surgery (CABG surgery), acute myocardial infarction, prevention of intimal hyperplasia following percutaneous transluminal coronary angioplasty (PTCA), prevention of intimal hyperplasia following stent placement, atherosclerosis, COPD, asthma, rheumatoid arthritis, osteoarthritis, Crohn's disease, inflammatory bowel disease (IBD), psoriasis, diabetes, stroke, Parkinson's disease, Alzheimer's disease, systemic lupus erythematosus, and vasculitis, in particular Wegener's granulomatosis, to be particularly useful for treatment with the JNK inhibitors of the present invention.

A person skilled in the art will readily realize that the above mentioned disease states and disorders may belong to more than one of the above mentioned disease classes. For example, bronchial carcinoma is certainly not only a proliferative disease but would also belong in the group of diseases of the respiratory system including lung diseases. Thus, the above mentioned classification of individual diseases is not considered to be limiting or concluding but is considered to of exemplary nature only. It does not preclude that individual disease states recited in one class are factually also suitable examples for the application of the JNK inhibitors of the present invention as treatment in another class of disease states. A person skilled in the art will readily be capable of assigning the different disease states and disorders to matching classifications.

Finally, as mentioned above, the present invention contemplates the use of a JNK inhibitor as defined herein for the treatment of various diseases states and disorders. The present invention does not contemplate to use the JNK inhibitors as defined herein for immunizing non-human animals, e.g. for the production of monoclonal antibodies. Such methods are herein not considered to be methods for treatment of the animal body by therapy.

All references cited herein are herewith incorporated by reference.

Examples

In the following, particular examples illustrating various embodiments and aspects of the invention are presented. However, the present invention shall not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become readily apparent to those skilled in the art from the foregoing description, accompanying figures and the examples below. All such modifications fall within the scope of the appended claims.

Example 1: Synthesis of JNK inhibitor SEQ ID NO: 172

As illustrative example, synthesis of the JNK inhibitor with SEQ ID NO: 172 is set out below. A person skilled in the art will know that said synthesis may also be used for and easily adapted to the synthesis of any other JNK inhibitor according to the present invention.

The JNK inhibitor with SEQ ID NO: 172 was manufactured by solid-phase peptide synthesis using the Fmoc (9-fluorenylmethyloxycarbonyl) strategy. The linker between the peptide and the resin was the Rink amide linker (p-[Fmoc-2,3-dimethoxybenzyl]-phenoxyacetic acid). The peptide was synthesized by successive Fmoc deprotection and Fmoc-amino acid coupling cycles. At the end of the synthesis, the completed peptide was cleaved by trifluoroacetic acid (TFA) directly to yield the crude C-terminal amide, which was then purified by preparative reverse phase HPLC. The purified fractions were pooled in a homogeneous batch that is treated by ion exchange chromatography to obtain its acetate salt. The peptide was then freeze-dried.

1.1 Solid Phase Synthesis of the Peptide

Except when noted, the manufacturing took place at room temperature (22°C ± 7°C) in an air-filtered environment. The scale of synthesis was 0.7 mmoles of the starting amino acid on the resin, for an expected yield of about 1g of purified peptide. Synthesis was performed manually in a 30–50 mL reactor equipped with a fritted disk with mechanical stirring and/or nitrogen bubbling.

1.2 Preparation of the resin

The p-methylbenzhydrylamide resin (MBHA-resin) was first washed with dichloromethane/ dimethylformamide/diisoproplyethylamine under nitrogen. The washed resin was then coupled to the Rink amide linker (p-[Fmox-2,4-dimethoxybenzyl]-phenoxyacetic acid) in PyBOB(benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate)/ diisopropyl-ethylamine/1-hydroxybenzotriazole to yield Fmoc-Rink amide-MBHA resin.

1.3 Coupling of Amino Acids

Amino acids were coupled to the resin using the following cycle:

The Fmoc-Rink amide-MBHA resin was deprotected by washing it in 35% (v/v) piperidine/dimethylformamide, followed by dimethylformamide. The deprotection reaction took approximately 16 minutes. Fmoc-protected amino acids (e.g., 2 eq of amino acid and HOBt (1-hydroxybenzotriazole) in dimethylformamide/dichloromethane (50/50) were added to the resin followed by addition of 2 eq of the coupling agent diisopropylcarbodiimide (DIC). The coupling reaction took from one hour to overnight depending upon the respective amino acid being added. Volumes were calculated on a basis of 0.5 mL/100mg of peptide-resin and adjusted after each cycle. After coupling, the resin was washed 3 times with DMF. Completeness of coupling was tested by the ninhydrin test (or Kaiser test 1) on primary amines and the chloranyl test 2 on secondary amines. On some occasions, the chloranyl test may be associated with a ninhydrin test as a security control. In case the coupling test indicated incompleteness of reaction, coupling was repeated with a lower excess (0.5-1 eq) of amino acid, PYBOP, HOBT in dimethylformamide/dichloromethane and diisopropylethylamine. Functionality of the resin was measured and generally 0.6–0.2 meq/g, depending on the original loading of the resin. After the last amino acid has been coupled, the peptide-resin was deprotected as usual and then washed 5 times with DCM before drying in an oven under vacuum at 30°C. After the peptide-resin had dried, the yield of the solid-phase synthesis was calculated as the ratio of the weight increase of the peptide resin compared to the theoretical weight increase calculated from the initial loading of the resin. The yield may be close to 100%.

1.4 Cleavage And Deprotection

The peptide was cleaved from the resin in a mixture of trifluoroacetic acid/1,2-ethanedthiol/ thioanisole/water/phenol (88/2.2/4.4/4.4/7 v/v), also called TFA/K reagent, for 4 hours at room temperature. The reaction volume was 1mL/100mg of peptide resin. During addition of the resin to the reagent, the mixture temperature was regulated to stay below 30°C.

1.5 Extraction of the peptide from the resin:

The peptide was extracted from the resin by filtration through a fritted disc. After concentration on a rotavapor to 1/3 of its volume, the peptide was precipitated by cold t-butyl methyl ether and filtered. The crude peptide was then dried under vacuum at 30°C.

1.6 Preparative HPLC Purification:

The crude peptide was then purified by reverse-phase HPLC to a purity of ≥95%. The purified fractions were concentrated on a rotavaporator and freeze-dried.

1.7 Ion Exchange Chromatography

The concentrated freeze-dried pools of purified peptide with the sequence of SEQ ID NO: 172 was dissolved in water and purified by ion exchange chromatography on Dowex acetate, 50–100 mesh resin.

The required starting reagents for the synthesis were :

	CAS Registry Number	Chemical Name	Molecular Weight
Fmoc-Rink amide linker	145069-56-3	p-[Fmoc-2,4-dimethoxybenzyl]-phenoxyacetic acid	539.6
Fmoc-D-Ala-OH, H_2O	79990-15-1	N-alpha-Fmoc-D-alanine	311.3
Fmoc-Arg(Pbf)-OH	154445-77-9	N-alpha-Fmoc-N [2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl]-arginine	648.8
Fmoc-D-Arg(Pbf)-OH	187618-60-6	N-alpha-Fmoc-N [2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl]-D-arginine	648.8
Fmoc-Asn(Trt)-OH	132388-59-1	N-alpha-Fmoc-N- -trityl-asparagine	596.7

Fmoc-Gln(Trt)-OH	132327-80-1	N-alpha-Fmoc-N- -trityl-glutamine	610.7
Fmoc-Leu-OH	35661-60-0	N-alpha-Fmoc-leucine	353.4
Fmoc-Lys(Boc)-OH	71989-26-9	N-alpha-Fmoc-N -Boc-lysine	468.5
Fmoc-D-Lys(Boc)-OH	143824-78-6	N-alpha-Fmoc-N -Boc-D-lysine	468.5
Fmoc-D-Phe-OH	86123-10-6	N-alpha-Fmoc-D-phenylalanine	387.4
Fmoc-Pro-OH	71989-31-6	N-alpha-Fmoc-proline	337.4
Fmoc-Thr(tBu)-OH	71989-35-0	N-alpha-Fmoc-O-t-butyl-threonine	397.5

Other JNK inhibitors of the present invention may be prepared in similar manner.

Example 2: Inhibitory efficacy of selected JNK inhibitors according to the present invention

In the following a standard operating procedure will be set forth describing how the Inhibitory efficacy of JNK inhibitors according to the present invention was measured. The method allows to measure in vitro, in a non radioactive standardized assay, the ability of a candidate compound to decrease the phosphorylation of the c-Jun specific substrate by JNK. Moreover, it will be illustrated how to determine the inhibitory effect (IC50) and the Ki of a chosen compound for JNK. The method is suitable to verify whether a candidate compound does or does not inhibit JNK activity. and a person skilled in the art will certainly understand how to adapt the below methods for his specific purposes and needs.

2.1 Material

AlphaScreen reagent and plate:

- His-JNK1 (ref 14-327, Upstate, 10 µg in 100 µl: concentration: 2.2 µM) 5nM final

- His-JNK2 (ref 14-329, Upstate, 10 µg in 100 µl: concentration: 2 µM) 5nM final
- His-JNK3 (ref 14-501, Upstate, 10 µg in 100 µl: concentration: 1.88 µM) 5nM final
- Anti-Phospho-cJun (ref 06-828, Upstate, lot DAM1503356, concentration: 44.5 µM) 10nM final
- Biotin-cJun (29-67):

sequence: Biotin – SNPKILKQSMTLNLADPVGSLKPHLRAKNSDLLTSPDVG (SEQ ID NO: 198), lot 100509 (mw 4382.11, P 99.28%) dissolved in H_2O, concentration: 10 mM) 30nM final

- ATP (ref AS001A, Invitrogen, lot 50860B, concentration 100 mM)) 5 µM final
- SAD beads (ref 6760617M, PerkinElmer, lot 540-460-A, concentration 5mg/ml) 20 µg/ml final
- AprotA beads (ref 6760617M, PerkinElmer, lot 540-460-A, concentration 5mg/ml) 20 µg/ml final
- Optiplate 384well white plate (ref 6007299, PerkinElmer, lot 654280/2008)
- 96well plate for peptide dilution (ref 82.1581, Sarstedt)
- TopSeals-A (ref 6005185, Perkin Elmer, Lot 65673)
- Bioluminescent energy transfer reading

- The bioluminescent energy transfer was read on the Fusion Alpha Plate reader (Perkin Elmer).

Pipette:

- An electronic EDP3 pipette 20-300 (Ref 17007243; Rainin) was used to fill in the plate with the Enzme-Antibody mix, the Subtrate-ATP mix and the Beads.
- A PIPETMAN® Ultra multichannel 8X20 (Ref 21040; Gilson) was used to fill in the plate with the inhibitory compounds.

Buffer and solutions

- Kinase Buffer: 20mM Tris-base pH 7.4, 10mM $MgCl_2$, 1mM DTT, 100µM Na_3VO_4, 0.01% Tween, (1% DMSO)
- Stop Buffer: 20mM Tris-base pH 7.4, 200mM NaCl, 80mM EDTA-K (pH de 8 with KOH instead of NaOH), 0.3% BSA
- JNK dilution Kinase buffer: 50mM Tris-base pH 7.4, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.1% β–mercaptoethanol.

2.2 Method

To assess inhibitory effect of the peptides, a standard AlphaScreen assay (see for example Guenat et al. J Biomol Screen, 2006; 11: pages 1015-1026) was performed. The different components were prepared and subsequently mixed as indicated. The plates were sealed and incubated as following:

5 µl	JNK + Antibody	
5 µl	TP kinase + / - inhibitor	Pre-incubation 30 min
5 µl	Biotin-cJun + ATP	Incubation 60 min at 24°C
10 µl	Beads SAD + A protA	Incubation 60 min in the dark at 24°C

To avoid contamination, the mixes were added with the pipette in different corner of the well. After the filling in of the plate with each mix, the plate was tapped (Keep one side fix and let the opposite side tap the table) to let the mix go down the walls of the wells.

The bioluminescent energy transfer was read on the Fusion Alpha Plate reader (Perkin Elmer).

All compounds should at least be tested in triplicate in 3 independent experiments for each isoform of JNK. Possibly concentrations of the compounds to be tested were 0, 0.03 nM, 0.1 nM, 0.3 nM, 1 nM, 3 nM, 10 nM, 30 nM, 100 nM, 300 nM, 1 µM, 3 µM, 10 µ M, 30 µM, and 100 µM. Controls were samples either without JNK or without substrate (c-Jun).

Mix preparation

JNK1, JNK2 and JNK3 5nM

Biotin-cJun 30 nM

ATP 5 µM; Anti phospho-cJun (S63) 10nM

Bille SAD/AprotA 20 µg/ml

Antibody [final] = 10nM (anti Phospho cJun (S63))

Detection part : [Mix] X5 (5 µl in final volume of 25 µl)

[Stock] = 44.5 µM (ref 06-828, Upstate, Lot DAM1503356)

10 nM → 50nM in Kinase Buffer

JNK1, JNK2 and JNK3 [final] = 5nM

Reaction part : [Mix] X3 (5 µl in final volume of 15 µl)

[Stock] = 2.2 µM for JNK1 (ref 14-327, Upstate, lot D7KN022CU)

2.0 µM for JNK2 (ref 14-329, Upstate, lot 33221CU)

1.88 µM for JNK3 (ref 14-501, Upstate, lot D7CN041CU)

5 nM → 15nM in Antibody Buffer

Inhibitor:

Reaction part : [Mix] X3 (5 µl in final volume of 15 µl)

[Stock] = 10 mM

100 µM	→	300 µM in Kinase Buffer
30 µM	→	90 µM in Kinase Buffer
10 µM	→	30 µM in Kinase Buffer
...		
0.03 nM	→	0.09 nM in Kinase Buffer
And 0 nM	→	Kinase Buffer

Two series of 10 times serial dilutions were performed in a 96 well plate, one beginning with 300 µM to 0 nM, the second with 90 µM to 0.03 nM. The peptides are added in the 384 plates with an 8 channels multipipette (ref F14401, Gilson, 8X20).

ATP [final] = 5 µM

Reaction part : [Mix] X3 (5 µl in final volume of 15 µl)

[Stock] = 100 mM (ref AS001A, Invitrogen, lot 50860B)

5 µM → 15 µM in Kinase Buffer

Biotin c-Jun [final] = 30nM

Reaction part : [Mix] X3 (5 µl in final volume of 15 µl)

[Stock] = 10 mM

30 nM → 30nM in ATP Buffer

Beads SAD / A ProtA [final] = 20 µg/ml (Light sensitive)

Detection part : [Mix] X 2.5 (10 µl in final volume of 25 µl)

[Stock] = 5 mg/ml → 20 µg/ml 50 µg/ml in STOP Buffer

Mix in the dark room (green Light) or in the darkness.

Analysis of the IC50 curves:

The analysis was performed by the GraphPad Prism4 software with the following equation: Sigmoidal dose-response (No constraint).

Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)))

The outliers data were avoided using Grugg's test.

Comparison of the IC50:

The analysis was performed by the GraphPad Prism4 software with the following test: One way ANOVA test followed by a Tukey's Multiple Comparison Test. $P<0.05$ was considerate as significant.

The Km of the ATP for JNK and the Km of biotin-cJun specific peptide were determined in the report AlphaScreen standardization assay

The mathematical relation between Ki and IC50 (Ki = IC50 / (1 + ([Substrate] / Km of the substrate)) may be used to calculate the Ki values.

Example 3: Internalization experiments and analysis

3.1 Materials and Methods for uptake experiments

a) Cell line:

The cell line used for this experiment was HL-60 (Ref CCL-240, ATCC, Lot 116523)

b) Culture medium and plates

RPMI (Ref 21875-091, Invitrogen, Lot 8296) or DMEM (Ref 41965, Invitrogen, Lot 13481) complemented on 05.05.2008 with:

10% FBS (Ref A64906-0098, PAA, Lot A15-151): decomplemented at 56°C, 30 min, on 04.04.2008.

1mM Sodium Pyruvate (Ref S8636, Sigma, Lot 56K2386)

Penicillin (100 unit/ml)/Streptomycin (100µg/ml) (Ref P4333, Sigma, Lot 106K2321)

PBS 10X (Ref 70011, Invitrogen, Lot 8277): diluted to 1X with sterile H_2O

Trypsine-0.05% EDTA (Ref L-11660, PAA, Lot L66007-1194)

6 well culture plates (Ref 140675, Nunc, Lot 102613)

24 well culture plates (Ref 142475, Nunc, Lot 095849)

96 well culture plates (Ref 167008, Nunc, Lot 083310)

96 well plates for protein dosing (Ref 82.1581, Sarstedt)

96 well plates for fluorescence measurement (Ref 6005279, Perkin Elmer)

c) Solutions

Poly-D-lysine coating solution (Sigma P9011 Lot 095K5104): 25µg/ml final diluted in PBS 1x

Acidic wash buffer: 0.2M Glycin, 0.15M NaCl, pH 3.0

Ripa lysis buffer: 10mM NaH_2PO_4 pH 7.2, 150mM NaCl, 1% Triton X-100, 1mM EDTA pH 8.0, 200µM Na_3VO_2, 0.1% SDS, 1X protease inhibitor cocktail (Ref 11873580001, Roche, Lot 13732700)

d) Microscopy and fluorescence plate reader

Cells were observed and counted using an inverted microscope (Axiovert 40 CFL; Zeiss; 20X).

The fluorescence was read with the Fusion Alpha Plate reader (Perkin Elmer).

e) Method

FITC marked peptide internalization was studied on suspension cells. Cells were plated into poly-DL-lysine coated dishes at a concentration of 1 x 10^6 cells/ml. Plates were then incubated for 24 h at 37 °C, 5 % CO_2 and 100% relative humidity prior to the addition of a known concentration of peptide. After peptide addition, the cells were incubated 30 min, 1, 6 or 24 h at 37 °C, 5 % CO_2 and 100 % relative humidity. Cells were then washed twice with an acidic buffer (Glycin 0.2 M, NaCl 0.15 M, pH 3.0) in order to remove the cell-surface adsorbed peptide (see Kameyama et al., (2007), *Biopolymers,* 88, 98-107). The acidic buffer was used as peptides rich in basic amino acids adsorb strongly on the cell surfaces, which often results in overestimation of internalized peptide. The cell wash using an acidic buffer was thus employed to remove the cell-surface adsorbed peptides. The acid wash was carried out in determining cellular uptake of Fab/cell-permeating peptide conjugates, followed by two PBS washes. Cells were broken by the addition of the RIPA lysis buffer. The relative amount of internalized peptide was then determined by fluorescence after background subtraction and protein content normalization.

The steps are thus:
1. Cell culture
2. Acidic wash and cellular extracts
3. Analysis of peptide internalization with a fluorescence plate reader

f) Cell culture and peptide treatment

The 6 well culture plates are coated with 3 ml of Poly-D-Lys (Sigma P9011; 25 µg/ml in PBS), the 24 well plates with 600 µl and the 96 well plates with 125 µl and incubated for 4 h at 37°C, CO_2 5 % and 100 % relative humidity.

After 4 hours the dishes were washed twice with 3.5ml PBS, 700 µl or 150 µl PBS for the 6, 24 or 96 well plates, respectively.

The cells were plated into the dishes in 2.4 ml medium (RPMI) at plating densities of 1'000'000 cells/ml for suspension cells. After inoculation, the plates were incubated at 37°C, 5 % CO_2 and 100 % relative humidity for 24 hours prior to the addition of the peptide. Adherent cells should be at a density of 90-95% the day of treatment and were plated in DMEM :

well	Surface of culture (cm^2)	Medium	Nb adherent cells	Nb suspension cells
96 well	0.3	100 – 200 µl	8'000 - 30'000	100'000
24 well	2	500 – 1000 µl	100'000 - 200'000	500'000- 1'000'000
35mm (P35) / 6 well	10	2,4 ml	250'000 - 2'100'000	2'400'000
60mm (P60)	20	3,5 ml	$15 * 10^5$	1'000'000/ml
10cm (P100)	60	10 ml	$15\text{-}60 * 10^5$	

The cells were treated with the desired concentration of FITC labeled peptide (stock solution at a concentration of 10 mM in H_2O).

Following peptide addition, the cells were incubated 0 to 24 hours (e.g. 30 min, 1, 6 or 24 hours) at 37 °C, CO_2 5 % and 100 % relative humidity.

Acidic wash and cellular extracts :

The extracts were cooled on ice.

Suspension cells (or cells, which don attach well to the dish):

Transfer the cells in « Falcon 15 ml ». To recover the maximum of cells, wash the dish with 1 ml of PBS.

Harvest the cells 2 min at 2400 rpm max.

Suspend the cells in 1 ml cold PBS.
Transfer the cells into a coated "Eppendorf tube" (coated with 1ml of poly D-Lys for 4hours and washed twice with 1ml PBS).
Wash three times with 1 ml of cold acidic wash buffer and centrifuge 2 min at 2400 rpm max.
Beware of the spreading of the cells in the "eppendorf".
Wash twice with 1 ml cold PBS to neutralize.
Add 50 µl of lysis RIPA Buffer.
Incubate 30 min-1h on ice with agitation.
Adherent cells:
Wash three times with 3 ml, 1 ml or 200 µl (for 6, 24 or 96 well plates, respectively) of cold acidic wash buffer. Beware of the cells who detach from the dish.
Wash twice with 1 ml cold PBS (for 6, 24 or 96 well plates, respectively) to neutralize.
Add 50 µl of lysis RIPA buffer.
Incubate 30 min-1h on ice with agitation.
Scrap the cells with a cold scrapper. The 24 and 96 well plates were directly centrifuged at 4000rpm at 4° for 15min to remove the cellular debris. Then the supernatants (100 or 50ml respectively for the 24 or 96 well plates) were directly transferred in a dark 96 well plated. The plates were read by a fluorescence plate reader (Fusion Alpha, Perkin Elmer).
Transfer the lysate in a coated "eppendorf" (coated with 1ml of poly D-Lys for 4hours and wash twice with 1ml PBS).
The lysed cells were then centrifuged 30 min at 10000 g at 4 °C to remove the cellular debris. Remove the supernatant and store it at -80 °C in a coated "Eppendorf tube" (coated with 1 ml of poly D-Lys for 4 hours and washed twice with 1 ml PBS).

Analysis of peptide internalization with a fluorescence plate reader :

The content of each protein extract was determined by a standard BCA assay (Kit N°23225, Pierce), following the instructions of the manufacturer.
The relative fluorescence of each sample is determined after reading 10 µl of each sample in a fluorescence plate reader (Fusion Alpha, Perkin Elmer), background subtraction and normalization by protein concentration.

3.2 Uptake experiments

The time dependant internalization (uptake) of FITC-labeled TAT derived transporter constructs into cells of the HL-60 cell line was carried out as described above using sequences transporter peptides of SEQ ID NOs: 52-96, 43, and 45-47. These sequences are listed below in Table 4.

Table 4:
Transporter sequence tested in uptake experiments

SEQ ID NO:	peptide No: abbreviation in Figure 6											
46	r3-L-TAT	H2N	dR	K	K	R	dR	Q	R	R	dR	CONH2
52	1	H2N	dR	A	K	R	dR	Q	R	R	dR	CONH2
53	2	H2N	dR	K	A	R	dR	Q	R	R	dR	CONH2
54	3	H2N	dR	K	K	A	dR	Q	R	R	dR	CONH2
55	4	H2N	dR	K	K	R	dR	A	R	R	dR	CONH2
56	5	H2N	dR	K	K	R	dR	Q	A	R	dR	CONH2
57	6	H2N	dR	K	K	R	dR	Q	R	A	dR	CONH2
58	7	H2N	dR	D	K	R	dR	Q	R	R	dR	CONH2
59	8	H2N	dR	K	D	R	dR	Q	R	R	dR	CONH2
60	9	H2N	dR	K	K	D	dR	Q	R	R	dR	CONH2
61	10	H2N	dR	K	K	R	dR	D	R	R	dR	CONH2
62	11	H2N	dR	K	K	R	dR	Q	D	R	dR	CONH2
63	12	H2N	dR	K	K	R	dR	Q	R	D	dR	CONH2
64	13	H2N	dR	E	K	R	dR	Q	R	R	dR	CONH2
65	14	H2N	dR	K	E	R	dR	Q	R	R	dR	CONH2
66	15	H2N	dR	K	K	E	dR	Q	R	R	dR	CONH2
67	16	H2N	dR	K	K	R	dR	E	R	R	dR	CONH2
68	17	H2N	dR	K	K	R	dR	Q	E	R	dR	CONH2
69	18	H2N	dR	K	K	R	dR	Q	R	E	dR	CONH2
70	19	H2N	dR	F	K	R	dR	Q	R	R	dR	CONH2
71	20	H2N	dR	K	F	R	dR	Q	R	R	dR	CONH2
72	21	H2N	dR	K	K	F	dR	Q	R	R	dR	CONH2
73	22	H2N	dR	K	K	R	dR	F	R	R	dR	CONH2
74	23	H2N	dR	K	K	R	dR	Q	F	R	dR	CONH2
75	24	H2N	dR	K	K	R	dR	Q	R	F	dR	CONH2
76	25	H2N	dR	R	K	R	dR	Q	R	R	dR	CONH2
77	26	H2N	dR	K	R	R	dR	Q	R	R	dR	CONH2
78	27	H2N	dR	K	K	K	dR	Q	R	R	dR	CONH2
79	28	H2N	dR	K	K	R	dR	R	R	R	dR	CONH2
80	29	H2N	dR	K	K	R	dR	Q	K	R	dR	CONH2
81	30	H2N	dR	K	K	R	dR	Q	R	K	dR	CONH2
82	31	H2N	dR	H	K	R	dR	Q	R	R	dR	CONH2
83	32	H2N	dR	K	H	R	dR	Q	R	R	dR	CONH2
84	33	H2N	dR	K	K	H	dR	Q	R	R	dR	CONH2
85	34	H2N	dR	K	K	R	dR	H	R	R	dR	CONH2
86	35	H2N	dR	K	K	R	dR	Q	H	R	dR	CONH2
87	36	H2N	dR	K	K	R	dR	Q	R	H	dR	CONH2
88	37	H2N	dR	I	K	R	dR	Q	R	R	dR	CONH2
89	38	H2N	dR	K	I	R	dR	Q	R	R	dR	CONH2
90	39	H2N	dR	K	K	I	dR	Q	R	R	dR	CONH2
91	40	H2N	dR	K	K	R	dR	I	R	R	dR	CONH2
92	41	H2N	dR	K	K	R	dR	Q	I	R	dR	CONH2
93	42	H2N	dR	K	K	R	dR	Q	R	I	dR	CONH2
94	43	H2N	dR	L	K	R	dR	Q	R	R	dR	CONH2
45	44 (D-TAT)	H2N	dR	dR	dR	dQ	dR	dR	dK	dK	dR	CONH2

47	45 (r3-L-TATi)	H2N	dR	R	R	Q	dR	R	K	K	dR	CONH2
46	46 (r3-L-TAT)	H2N	dR	K	K	R	dR	Q	R	R	dR	CONH2
43	47 (L-TAT)	H2N	R	K	K	R	R	Q	R	R	R	CONH2
99	48	H2N	dR	K	K	R	dR	Q	R	L	dR	CONH2
100	49	H2N	dR	M	K	R	dR	Q	R	R	dR	CONH2
101	50	H2N	dR	K	M	R	dR	Q	R	R	dR	CONH2
102	51	H2N	dR	K	K	M	dR	Q	R	R	dR	CONH2
103	52	H2N	dR	K	K	R	dR	M	R	R	dR	CONH2
104	53	H2N	dR	K	K	R	dR	Q	M	R	dR	CONH2
105	54	H2N	dR	K	K	R	dR	Q	R	M	dR	CONH2
106	55	H2N	dR	N	K	R	dR	Q	R	R	dR	CONH2
107	56	H2N	dR	K	N	R	dR	Q	R	R	dR	CONH2
108	57	H2N	dR	K	K	N	dR	Q	R	R	dR	CONH2
109	58	H2N	dR	K	K	R	dR	N	R	R	dR	CONH2
110	59	H2N	dR	K	K	R	dR	Q	N	R	dR	CONH2
111	60	H2N	dR	K	K	R	dR	Q	R	N	dR	CONH2
112	61	H2N	dR	Q	K	R	dR	Q	R	R	dR	CONH2
113	62	H2N	dR	K	Q	R	dR	Q	R	R	dR	CONH2
114	63	H2N	dR	K	K	Q	dR	Q	R	R	dR	CONH2
115	64	H2N	dR	K	K	R	dR	K	R	R	dR	CONH2
116	65	H2N	dR	K	K	R	dR	Q	Q	R	dR	CONH2
117	66	H2N	dR	K	K	R	dR	Q	R	Q	dR	CONH2
118	67	H2N	dR	S	K	R	dR	Q	R	R	dR	CONH2
119	68	H2N	dR	K	S	R	dR	Q	R	R	dR	CONH2
120	69	H2N	dR	K	K	S	dR	Q	R	R	dR	CONH2
121	70	H2N	dR	K	K	R	dR	S	R	R	dR	CONH2
122	71	H2N	dR	K	K	R	dR	Q	S	R	dR	CONH2
123	72	H2N	dR	K	K	R	dR	Q	R	S	dR	CONH2
124	73	H2N	dR	T	K	R	dR	Q	R	R	dR	CONH2
125	74	H2N	dR	K	T	R	dR	Q	R	R	dR	CONH2
126	75	H2N	dR	K	K	T	dR	Q	R	R	dR	CONH2
127	76	H2N	dR	K	K	R	dR	T	R	R	dR	CONH2
128	77	H2N	dR	K	K	R	dR	Q	T	R	dR	CONH2
129	78	H2N	dR	K	K	R	dR	Q	R	T	dR	CONH2
130	79	H2N	dR	V	K	R	dR	Q	R	R	dR	CONH2
131	80	H2N	dR	K	V	R	dR	Q	R	R	dR	CONH2
132	81	H2N	dR	K	K	V	dR	Q	R	R	dR	CONH2
133	82	H2N	dR	K	K	R	dR	V	R	R	dR	CONH2
134	83	H2N	dR	K	K	R	dR	Q	V	R	dR	CONH2
135	84	H2N	dR	K	K	R	dR	Q	R	V	dR	CONH2
136	85	H2N	dR	W	K	R	dR	Q	R	R	dR	CONH2
137	86	H2N	dR	K	W	R	dR	Q	R	R	dR	CONH2
138	87	H2N	dR	K	K	W	dR	Q	R	R	dR	CONH2
139	88	H2N	dR	K	K	R	dR	W	R	R	dR	CONH2
140	89	H2N	dR	K	K	R	dR	Q	W	R	dR	CONH2
141	90	H2N	dR	K	K	R	dR	Q	R	W	dR	CONH2
142	91	H2N	dR	Y	K	R	dR	Q	R	R	dR	CONH2
143	92	H2N	dR	K	Y	R	dR	Q	R	R	dR	CONH2
144	93	H2N	dR	K	K	Y	dR	Q	R	R	dR	CONH2
145	94	H2N	dR	K	K	R	dR	Y	R	R	dR	CONH2
146	95	H2N	dR	K	K	R	dR	Q	Y	R	dR	CONH2

| 147 | 96 | H2N | dR | K | K | R | dR | Q | R | Y | dR | CONH2 |

In the above table D amino acids are indicated by a small "d" prior to the respective amino acid residue (e.g. dR = D-Arg).

For a few sequences synthesis failed in the first approach unfortunately due to technical reasons. These sequences are abbreviated in Figure 6 as 1, 2, 3, 4, 5, 6, 7, 8, 43, 52, 53, 54, 55, 56, 57, 85, 86, 87, 88, 89, and 90. However, the remaining sequences were used in the internalization experiments.

The results are shown in Figure 6.

As can be seen in Figure 6, after 24 hours incubation, all transporters with the consensus sequence rXXXrXXXr (SEQ ID NO: 31) showed a higher internalization capability than the L-TAT transporter (SEQ ID NO: 43). Hela cells were incubated 24hours in 96well plate with 10mM of the r3-L-TAT-derived transporters. The cells were then washed twice with an acidic buffer (0.2M Glycin, 0.15M NaCl, pH 3.0) and twice with PBS. Cells were broken by the addition of RIPA lysis buffer. The relative amount of internalized peptide was then determined by reading the fluorescence intensity (Fusion Alpha plate reader; PerkinElmer) of each extract followed by background subtraction

As can be seen in Figure 6, one positions appears to be critical for highest transporter activity and for improved kinetics of transport activity: Y in position 2 (peptide N°91 corresponding to SEQ ID NO: 142).

The conclusion of this experiment is as follows:

- After 24 hours incubation, all transporters with the consensus sequence rXXXrXXXr (SEQ ID NO: 31) (see Table 2 for a selection of possible sequences) showed a higher internalization capability than the L-TAT transporter (SEQ ID NO: 43) (Figure 6). Those results fully validate the consensus sequence rXXXrXXXr (SEQ ID NO: 31).
- One position is critical for highest transporter activity and (Figure 6): Y in position 2 (sequence 91 corresponding to SEQ ID NO: 142).

Accordingly, such TAT derived sequences as shown in Table 4 are preferred, which exhibit an Y in position 2, particularly when the sequence exhibits 9 aa and has the consensus sequence rXXXrXXXr (SEQ ID NO: 31).

Example 4: Measurement of cytokine and chemokine release

In the following the procedure will be set forth describing how the released amount of several human cytokines after ligand induced secretion from human cells (Blood, WBC, PBMC, purified primary lymphocytes, cell lines, ...) was measured.

The technique used is a Sandwich ELISA, which allows measuring the amount of antigen between two layers of antibodies (i.e. capture and detection antibody). The antigen to be measured must contain at least two antigenic sites capable of binding to antibody, since at least two antibodies act in the sandwich. Either monoclonal or polyclonal antibodies can be used as the capture and detection antibodies in Sandwich ELISA systems. Monoclonal antibodies recognize a single epitope that allows fine detection and quantification of small differences in antigen. A polyclonal is often used as the capture antibody to pull down as much of the antigen as possible. The advantage of Sandwich ELISA is that the sample does not have to be purified before analysis, and the assay can be very sensitive (up to 2 to 5 times more sensitive than direct or indirect).

The method may be used to determine the effect of the JNK inhibitors of the present invention in vitro/ cell culture. At non toxic doses, compound efficacy is indicated by the decrease of the cytokine levels (the variation of optical density (absorbance at 450 nm)) as compared to non-treated samples and is monitored by ELISA. Results are express in ng/ml.

4.1 Material

- 96 well plate:
  for collecting the supernatants (Ref 82.1581, Sarstedt)
  for ELISA (F96 maxisorp, Ref 442404, Nunc)
- TopSeal-A: 96well microplate seals (Ref 600585, PerkinElmer).
- ELISA reagent

  Coating buffer ELISA: 0.1M NaCarbonate pH 9.5 (= 7.13g $NaHCO_3$ (ref 71627, Fluka) + 1.59g Na_2CO_3 (ref 71345, Fluka) in 1 litre H2O, pH to 9.5 with NaOH concentrated)

  Wash buffer ELISA: PBS 1X + 0.01% Tween20. Prepare 1 litre PBS 1X (PBS10X: ref 70011, GIBCO) and add 100ul of Tween20 (ref P1379, Sigma) slowly while mixing with magnetic agitator)

Assay diluent: PBS 1X + 10% FBS (Ref A15-151, PAA, decomplemented at 56°C, 30 min).

DAKO TMB (ref S1599, DAKO): commercial substrate solution

Stop Solution: 1M H_3PO_4 (→ for 200ml = 177ml H_2O + 23ml H_3PO_4 85% (ref 345245, Aldrich).

- ELISA Kit (reagent for 20 plates)

  IFN-γ: Human IFN- ELISA set, BD OptEIA™ (ref 555142, DB).

  IL-1β: Human IL-1 ELISA set II, BD OptEIA™ (ref 557953, BD)

  IL-10 : Human IL-10 ELISA set II, BD OptEIA™ (ref 555157, DB).

  IL-12 : Human IL-12 (p70) ELISA set, BD OptEIA™ (ref 555183, DB).

  IL-15 : Human IL-15 ELISA Set, BD OptEIA™ (ref 559268, DB).

  IL-2: Human IL-2 ELISA set, BD OptEIA™ (ref 555190, DB).

  IL-4 : Human IL-4 ELISA set, BD OptEIA™ (ref 555194, DB).

  IL-5 : Human IL-5 ELISA set, BD OptEIA™ (ref 555202, DB).

  IL-6: Human IL-6 ELISA setI, BD OptEIA™ (ref 555220, DB).

  IL-8: Human IL-8 ELISA set, BD OptEIA™ (ref 555244, DB).

  MCP-1: Human MCP-1 ELISA set, BD OptEIA™ (ref 555179, BD)

  TNF-α: Kit human TNF ELISA set, BD OptEIA™ (ref 555212, DB).

- Absorbance reading: The absorbance was read on the Fusion Alpha Plate reader (Perkin Elmer).
- Repeating pipettes, digital pipettes or multichannel pipettes.

4.2 Method

Preparation of the samples

The samples are culture medium supernatant from cultured human cells (typically whole blood, WBC, PBMC, Purified subtype of WBC, cancerous cell lines). Remove any particulate material by centrifugation (400g 5min 4°C) and assay immediately or store samples at -20°C. Avoid repeated freeze-thaw cycles.

One hour before using, defrost the samples on ice and centrifuge them. At step 11, dilute the samples in assay diluent directly into the plate (add first assay diluent, then the samples and pipette up and down):

Preparation of Standard

After warming lyophilized standard to room temperature, carefully open vial to avoid loss of material. Reconstitute lyophilized standard with the proposed

volume of deionized water to yield a stock standard. Allow the standard to equilibrate for at least 15 minutes before making dilutions. Vortex gently to mix. After reconstitution, immediately aliquot standard stock in polypropylene vials at 50 µl per vial and freeze at -20°C for up to 6 months. If necessary, store at 2-8° C for up to 8 hours prior to aliquotting/freezing. Do not leave reconstituted standard at room temperature.

Immediately before use, prepare a ten point standard curve using 2-fold serial dilutions in reagent Diluent. A high standard of 4000 pg/ml is recommended.

Preparation of Detector Mix

One-step incubation of Biotin/SAv reagents. Add required volume of Detection Antibody to Assay Diluent. Within 15 minutes prior to use, add required quantity of Enzyme Reagent, vortex or mix well. For recommended dilutions, see lot-specific Instruction/Analysis Certificate. Discard any remaining Working Detector after use.

Coating with Capture Antibody

1. Coat the wells of a PVC microtiter plate with 100 µL per well of Capture Antibody diluted in Coating Buffer. For recommended antibody coating dilution, see lot-specific Instruction/Analysis Certificate.
2. Cover the plate with an adhesive plastic and incubate overnight at 4°C.
3. Remove the coating solution and wash the plate by filling the wells with 150µl wash buffer.
4. The solutions or washes are removed by flicking the plate over a sink.
5. Repeat the process two times for a total of three washes.
6. After the last wash, remove any remaining wash buffer by patting the plate on a paper towel.

Blocking

7. Block the remaining protein-binding sites in the coated wells by adding 100µl reagent Diluent per well.
8. Cover the plate with an adhesive plastic and incubate for 1 h at room temperature.
9. During the incubation, start preparing the standard.

Adding samples

10. Do one wash as in step 3 with 150µl of wash buffer. The plates are now ready for sample addition.
11. Add 50 µl of appropriately diluted samples in assay diluent to each well. For accurate quantitative results, always compare signal of unknown samples against those of a standard curve. Standards (triplicates) and blank must be run with each cytokine to ensure accuracy.
12. Cover the plate with an adhesive plastic and incubate for 2 h at room temperature.

Incubation with Detection Antibody and Secondary Antibody

13. Wash the plate four times with 150µl wash buffer like step 3.
14. Add 50 µl of detector MIX (detection antibody +Secondary Streptavidin-HRP antibody in assay diluent) to each well at recommended dilutions (see lot-specific Instruction/Analysis Certificate).
15. Cover the plate with an adhesive plastic and incubate for 1 h at room temperature light protect.
16. Wash the plate six times with 150µl wash buffer as in step 3.
17. Add 50 µl DAKO TMB solution to each well, incubate for 15-20 min at room temperature, in the dark, not sealed.
18. Add 50 µl of stop solution to each well. Gently tap the plate to ensure thorough mixing.
19. Mix the plate 5min at 500rpm on a plate mixer.
20. Read the optical density at 450 nm. (Program: Cytokine_ELISA on Fusion Alpha Plate reader).

Data analysis

Average the triplicate readings for each standard control and each sample. Subtract the average zero standard optical density (O.D). Create a standard curve plotting the log of the cytokine concentration versus the log of the O.D and the best fit line can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. A standard curve should be generated for each set of samples

assayed. The outliers data were avoided using Grugg's test. Then the data which weren't in the interval of two times the SD, were discard. The independent experiments are taken into account if the positive control showed data as previously observed. The independent experiments are pooled (N > 3).

The data are presented in pg/ml of cytokine release or in %, compared to the induced condition without inhibitor treatment.

Example 5: THP1 differentiation – stimulation for cytokine release

In the following the procedure will be set forth describing how cytokine production from human PMA differentiated THP1 cells challenged by LPS for 6h was induced in order to test the ability of JNK inhibitors of the present invention, in particular of a JNK inhibitor with SEQ ID NO: 172, to reduce stimulation-induced cytokine release. THP1 cells were stimulated ex-vivo by different ligands for the readout of cytokine release. At non toxic doses, JNK inhibitor efficacy is indicated by the decrease of the cytokine levels as compared to non-treated samples and is monitored by ELISA. The toxicity of the compound are evaluated by the reduction of a tretazolium salt (MTS) to formazan, giving a purple colour.

Procedure:

a. Material

- Cell Line: THP-1 (Ref TIB-202, ATCC, lot 57731475)
- Culture medium, reagent and plates

RPMI (Ref 21875-091, Invitrogen) complemented with:

10% FBS (Ref A15-151, PAA): decomplemented at 56°C, 30 min.

10mM Hepes (Ref H0887, Sigma)

50 M -mercaptoethanol (Ref 63690, Fluka : stock at 14.3M): add 560 l of 50mM aliquots in PBS stocked at -20°C)

1mM Sodium Pyruvate (Ref S8636, Sigma)

Penicilline (100unit/ml) / Streptomycine (100 g/ml) (Ref P4333, Sigma)

The RPMI medium is then filtrated with a 0.22 M filter (Ref SCGPU05RE, Millipore).

PBS 10X (Ref 70011, Invitrogen): diluted to 1X with sterile H_2O

DMSO: Ref 41444, Fluka

PMA (phorbol 12-myristate 13-acetate, Ref P1585, Sigma, concentration 1mM = 616.8ug/ml in DMSO at -20°C). Use directly at a final concentration of 100nM in RPMI (1ul in 10ml of medium).

LPS ultrapure (Lipopolysaccharide, Ref tlrl-eklps, Invivogen, concentration 5mg/ml): Stock solution of LPS: 3 g/ml in PBS at 4°C. Use directly to prepare a 4X concentrated solution of 40ng/ml in RPMI medium (min 1800 l /plate; for 5 plates: 125 l of LPS 3 g/ml + 9250 l RPMI).

96 well plate:

for adherent cell culture (Ref 167008, Nunc)

for collecting the supernatants (Ref 82.1581, Sarstedt)

for ELISA (F96 maxisorp, Ref 442404, Nunc)

Coating solutions: poly-D-lysine (Ref P9011, Sigma): 25 g/ml final diluted in PBS 1x

- ELISA reagent and kits

Coating buffer ELISA: 0.1M NaCarbonate pH 9.5 (= 7.13g $NaHCO_3$ (ref 71627, Fluka) + 1.59g Na_2CO_3 (ref 71345, Fluka) in 1 liter H2O, pH to 9.5 with NaOH concentrated)

Wash buffer ELISA: PBS 1X + 0.01% Tween20 (ref P1379, Sigma, lot 094K0052)(= prepare 1 liter PBS 1X and add 100ul of Tween20 slowly while mixing with magnetic agitator)

Assay diluent: PBS 1X + 10% FBS (Ref A15-151, PAA, decomplemented at 56°C, 30 min).

DAKO TMB (ref S1599, DAKO): commercial substrate solution

Stop Solution: 1M H_3PO_4 (→ for 200ml = 177ml H_2O + 23ml H_3PO_4 85% (ref 345245, Aldrich).

TNF- : Kit human TNF ELISA set, BD OptEIA (ref 555212, DB).

- Cytotoxicity measurement: CellTiter 96 reagent (ref G3581, Promega)
- Control compound: SP600125 (ref ALX-270-339-M025, Alexis, concentration: 20mM DMSO)
- Absorbance reading: The absorbance was read on the Fusion Alpha Plate reader (Perkin Elmer).
- Repeating pipettes, digital pipettes or multichannel pipettes.
- TopSeal-A: 96well microplate seals (Ref 600585, PerkinElmer).

b. Method

Well coating

The plates had been coated with 200 l of poly D-Lysine (1x) and incubated 2 hours at 37°C, CO_2 5% and 100% relative humidity.

Cell plating

After 2 hours the wells were washed twice with 200 l PBS 1X (use immediately or leave with 200 l of PBS 1X at 37°C till use, but no more than 3 days).

The cells were counted. The desired number of cells was taken and resuspended in the amount of media necessary to get a dilution of 1'000'000 cells/ml. 100nM of PMA was added to induce the differentiation of the THP1 from suspension monocytes to adherent macrophages. The cells were plated into the wells in 100 l medium at plating densities of 100'000cells/well. After inoculation, the plates were incubated at 37°C, 5% CO2 and 100% relative humidity 3 days to let them differentiate, prior to the addition of experimental drugs.

Cell treatment

After 3 days, the adherent cells were observed with the microscope. The media containing PMA was aspirated and replaced by 100 l of fresh RPMI media without PMA (no washing step with PBS 1X).

Experimental drug were prepared at the concentration of 10 mM in H_2O or DMSO and stored at -80°C. Prior to each daily use, one aliquot of JNK inhibitorwas defrost and diluted to reach a 4X concentrated solution (120 M) in RPMI medium and then to the desired concentration in RPMI. The SP600125 was diluted to reach a 4X concentrated solution (40 M) in RPMI medium and then to the desired concentration in RPMI containing 0.8% DMSO.

The plates were treated with 50 l of medium or a solution of 4X the final desired drug concentration (0, 100nM, 1, 3, 10 or 30 M final for JNK compound or at 0, 10, 100nM, 1, 3 or 10 M final for the SP600125 positive control). Following drug addition, the plates were incubated for an additional 1h at 37°C, 5% CO_2 and 100% relative humidity.

After 1hours, the secretion of TNF was induced by the addition of 50 l of a 4X concentrated dilution of LPS ultrapure (3ng/ml final).

Assay

After 6 hours, 100 l of the supernatant were transferred to new 96well plates. Those plates were sealed and stored at -20° till the analysis by ELISA (e.g. see example 4) of the secretion of the cytokines.

The cytotoxic effect of the compounds was evaluated by MTS absorbance (e.g. see example 4) and cells were observed using an inverted microscope (Axiovert 40 CFL; Zeiss; 10X).

Data analysis

Analyses of the data are performed as indicated in the ELISA (see example 4). Briefly, for ELISA: Average the triplicate readings for each standard control and each sample. Subtract the average zero standard optical density (O.D). Create a standard curve plotting the log of the cytokine concentration versus the log of the O.D and the best fit line can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. A standard curve should be generated for each set of samples assayed. The outliers data were avoid using Grugg's test. Then the data which weren't in the interval of two times the SD, were discard. The independent experiments are taken into account if the positive control showed data as previously observed. The independent experiments are pooled ($N > 3$).

For the Cytotoxicity effect evaluation: on each plate of each independent experiment taken into account for the cytokine release experiment analysis, the average of the absorbance of the medium alone was considerate as the background and subtracted to each absorbance value. The average of triplicate of the non treated cells of each compound was considerate as the 100% viability. The average of triplicate of each compound was normalized by its 100%. The outliers data were avoid using Grugg's test. Then the data which weren't in the interval of two times the SD, were discard. The independent experiments are pooled ($N > 3$).

All statistical comparisons of conditions were performed by the GraphPad Prism4 software with the following test: One way ANOVA test followed by a Tukey's Multiple Comparison Test. $P<0.05$ was considerate as significant..

Example 6: JNK inhibitor of SEQ ID NO: 172 and TNFα release in Primary Rat or human whole blood cells

Whole blood is collected from anesthetized rat or human healthy volunteers using a venipuncture connected to a pre-labeled vacuum tube containing sodium citrate. Tubes are gently mixed by inversion 7-8 times; and are then kept at RT until stimulation. JNK inhibitor of SEQ ID NO: 172_is prepared 6 times concentrated in PBS, and 30 µl/well of mix is added into 96-well plate. Whole blood is diluted by 1:2 in PBS and 120 µl of diluted blood is added in each well where either PBS alone or JNK inhibitor of SEQ ID NO: 172 has been previously added. Whole blood is incubated at 37°C; 85 rpm (Stuart Orbital incubator SI500) for 60 min. Activators (LPS) are the prepared,

30µl/well of LPS, 6 times concentrated. After 60min incubation, LPS is added to the blood, blood is mixed by pipetting up and down, and then kept for 4h under agitation (85rpm), at 37°C. After the 4h incubation, the plates are centrifuged at about 770g, 4°c for 15 min in a pre-cooled centrifuge. Supernatants are finally collected and kept at -20°c until cytokine measurement. Cytokine (IL-6, IL-2, IFNγ and TNFα) were then measured using standard Elisa kits (e.g. from R&D Systems: DuoSet Elisas; or from BD Biosciences: BD Opteia Set Elisa). Results are expressed as pg/ml of supernatant of the measured cytokine.

A similar experiment was conducted with PMA+ionomycin instead of LPS as activator/stimulant.

Example 7: Half-life of specific JNK inhibitors disclosed herein

The JNK inhibitors with the sequence of SEQ ID NOs: 196, 197, and 172 (0.1mM final concentration) were digested in human serum (10 and 50% in PBS 1x). The experiment was performed as described by Tugyi et al. (Proc Natl Acad Sci U S A, 2005, 413-418). . The remaining intact peptide was quantified by UPLC-MS. Stability was assessed for SEQ ID NOs: 196, 197, and 172 identically but in two separate assays. While the JNK inhibitor with SEQ ID NO: 196 was totally degraded into amino acids residues within 6 hours, the JNK inhibitor with SEQ ID NO: 172 was completely degraded only after 14 days. The JNK inhibitor with SEQ ID NO: 197 was still stable after 30 days.

Example 8: Dose-dependent inhibition by JNK inhibitor with sequence of SEQ ID NO: 172 of CD3/CD28-induced IL-2 release in rat primary T-cells

Control animal were sacrificed, lymph nodes (LN) were harvested and kept in complete RPMI medium. LN were smashed with complete RPMI on 70µm filter using a 5ml piston. A few drops of media were added to keep strainer wet. Cells were centrifuged for 7 min at 450g and 4°c. Pellet was resuspended in 5 ml fresh medium. Cells were passed again through cell strainer. An aliquot of cells was counted, while cells were centrifuged again 10min at 1400 rpm and 4°c. Cells were resupended in MACS buffer (80µl of MACS buffer per 10^7 cells). 10µl of anti-rat MHC microbeads were added per 10 million cells, cells were incubated for 15min at 4°-8°c. Cells were washed with 15ml MACS buffer and centrifuge for 7 min at 700g and 4°C. Pellet was resuspended in 500µl MACS buffer per 10^8 cells. One LS column was placed in the magnetic field of the MACS separator per animal. Column was first rinsed with 3 ml of MACS buffer. One tube was placed

below the column in ice to collect cells = T cells (negative selection so we collect what is eluted). Cell suspension was added and elute was collected on ice. Column was washed 3 times with 3mL MACS buffer. Eluted T cells were centrifuges for 7 min at 700g and 4°C. Resuspended cells were counted and plated at density of 200000cells/well in 100µl of complete medium. Plates were precoated the day before experiment with 2µg/mL of CD3 antibody, and the day of experiment plates were washed three times with PBS. Cells were treated with 100µl of (poly-)peptide JNK inhibitor (SEQ ID NO: 172), two times concentrated for 1h before ligand activation. After 1h of pretreatment with (poly-)peptide JNK inhibitor (SEQ ID NO: 172), cells were then stimulated with 2µg/mL of anti CD28 antibody for 24h. After 24h of stimulation, supernatant were collected and stored at -20°C until analysis. Cytokines were then measured using standard Elisa kits. Results are expressed as pg/ml of supernatant of the measured cytokine.

In a further experiment, essentially the same protocol as set forth above was used, but in addition to the (poly-)peptide JNK inhibitors with SEQ ID NO: 172, JNK inhibitors with the sequence of SEQ ID NO: 197 and the drug molecule SP600125 were also tested thus allowing to compare the effects of these inhibitors on the inhibition of CD3/CD28-induced IL-2 release.

Example 9: JNK inhibitor and TNFα/IL-2 release in human whole blood:

Whole blood from human healthy volunteers was collected using a venipuncture connected to a pre-labeled vacuum tube containing sodium citrate. Tubes are gently mixed by inversion 7-8 times; and are then kept at RT until stimulation. 350µl of RPMI + P/S were added into 1,2 ml-96-well plate. 10 times concentrated of SEQ ID NO: 172 was prepared in RPMI+P/S (50µl per well). 50µl was added into 1.2ml- 96 well plates. 50µl of whole blood was then added in each well where either medium alone or JNK inhibitor has been previously added. Whole blood was incubated at 37°C, 5% CO2 for 60 min. 50µl / well of ligands diluted in RPMI+ P/S was prepared, corresponding to the final dilution 10 times concentrated. After 60min of incubation, ligand was added; wells were then mixed by pipetting up and down the blood. Whole blood was incubated for 3 days at 37°C (wells were mixed by pipetting each well up and down once per day). At the end of incubation, plates were mixed and then centrifuged at 2500rpm, 4°C for 15 min in a pre-cooled centrifuge. Cytokine were then measured using standard Elisa kits. Results are expressed as pg/ml of supernatant of the measured cytokine.

A similar experiment was carried out with slight modifications. In the case of CD3/CD8 stimulation, CD3 antibody was coated at 2µg/mL in PBS overnight at 4°C. The day of experiment,

wells were washed three times with PBS and left in PBS until use at 37°C. CD28 antibody was added 1h after SEQ ID NO: 172 at final concentration of 2µg/mL; supernatants were collected after 3 days of stimulation.

Example 10: Anti-Inflammatory potency in a rat model of endotoxins induced uveitis (EIU)

The anti-inflammatory potency of the JNK inhibitor of SEQ ID NO: 172 was tested in albino rats following intravenous administration (EIU/LPS model). The aim of this study was to determine the effects of single intravenous injections of SEQ ID NO: 172 (0.015, 0.18, and 1.80 mg/kg) on the inflammatory response in an endotoxins-induced uveitis albino rat model and to compare these affects to those obtained with prior art JNK inhibitor of SEQ ID NO: 197 (2 mg/kg). As a further control served phosphate sodic dexamethasone.

Sixty (60) male Lewis rats were randomly divided into six (6) groups of ten (10) animals each. EIU was induced by footpad injection of lipopolysaccharide (LPS, 1 mg/kg). NaCl (0.9%), SEQ ID NO: 197 at 2 mg/kg and SEQ ID NO: 172 at three concentrations (1.80 mg/kg, 0.18 mg/kg and 0.015 mg/kg) were administered by intravenous injection. Phosphate sodic dexamethasone (20 µg/eye) was administered by sub-conjunctival injection in both eyes. 24 hours after LPS injection, inflammatory response was evaluated by clinical scoring.

The intensity of clinical ocular inflammation was scored on a scale from 0 to 4 for each eye:

Grade 0	no inflammation
Grade 1	slight iris and conjunctival vasodilation
Grade 2	moderate iris and conjunctival vasodilation with flare
Grade 3	intense iris and conjunctival vasodilation with flare
Grade 4	intense inflammatory reaction
(+1) fibrin formation and seclusion of pupils	

Twenty-four hours after LPS induction, clinical scores for the vehicle-treated rats were 3.6 ± 0.2 (mean ± SEM, n = 20) with a median of 4 (range, 2-5). A significant reduction ($p < 0.001$) in the severity of the ocular inflammation was detected 24 hours after induction and intravenous treatment with SEQ ID NO: 197 (2 mg/kg) (mean score: 2.2 ± 0.3, median: 2), corresponding to a 40% decrease of EIU scores compared with the score observed in vehicle group. Intravenous treatment with SEQ ID NO: 172, at approximately the same dose (1.80 mg/kg) reduced also significantly the severity of the ocular inflammation by 42% (mean score: 2.1 ± 0.3, median: 2, $p = 0.001$). The lower doses (0.18 and 0.015 mg/kg) reduced by 33% (mean score: 2.4 ± 0.3,

median: 2) and 36% (mean score: 2.3 ± 0.3, median: 2) the inflammation, respectively. The reduction was significant with $p < 0.001$.

A sub-conjunctival treatment with dexamethasone (20 µg/eye), used as positive control drug also significantly reduced the clinical scores by 79% (mean score: 0.8 ± 0.2, median: 0.5, $p < 0.001$).

Under these experimental conditions, it can be stated that a single intravenous injection of SEQ ID NO: 197 at 2 mg/kg partially prevented the endotoxin-induced inflammation observed in the anterior chamber. In comparison, SEQ ID NO: 172 intravenously injected at 0.015, 0.18, 1.80 mg/kg also reduced the endotoxin-induced inflammation in the anterior chamber.

Example 11: Dose-responsive effects after intravenous administration of JNK inhibitor after 14 days in a rat model of chronic established type II collagen arthritis

Rat collagen arthritis is an experimental model of polyarthritis that has been widely used for preclinical testing of numerous anti-arthritic agents that are either under preclinical or clinical investigation or are currently used as therapeutics in this disease. The hallmarks of this model are reliable onset and progression of robust, easily measurable polyarticular inflammation, marked cartilage destruction in association with pannus formation, and mild to moderate bone resorption and periosteal bone proliferation.

Itravenous (IV) efficacy of the JNK inhibitor of SEQ ID NO: 172 administered daily (QD) for 14 days (arthritis d1–14) for inhibition of the inflammation (paw swelling), cartilage destruction, and bone resorption that occurs in established type II collagen arthritis in rats was determined in said experimental model.

Animals (8/group for arthritis) were anesthetized with Isoflurane and injected with 300 µl of Freund's Incomplete Adjuvant (Difco, Detroit, MI) containing 2 mg/ml bovine type II collagen (Elastin Products, Owensville, Missouri) at the base of the tail and 2 sites on the back on days 0 and 6. On day 10 of the study (arthritis d0), onset of arthritis occurred and rats were randomized into treatment groups. Randomization into each group was done after ankle joint swelling was obviously established in at least one hind paw.

Female Lewis rats with established type II collagen arthritis were treated daily (QD) on arthritis days 1-14 by the intravenous (IV) route with vehicle (NaCl), SEQ ID NO: 172 (0.01, 0.1, 1, or 5

mg/kg), or the reference compound dexamethasone (Dex, 0.05 mg/kg). Animals were terminated on arthritis day 14. Efficacy evaluation was based on animal body weights, daily ankle caliper measurements, ankle diameter expressed as area under the curve (AUC), terminal hind paw weights, and histopathologic evaluation of ankles and knees of selected groups.

Scoring of Joints Collagen arthritic ankles and knees are given scores of 0-5 for inflammation, pannus formation and bone resorption according to the following criteria:

Knee and/or Ankle Inflammation

0	Normal
0.5	Minimal focal inflammation
1	Minimal infiltration of inflammatory cells in synovium/periarticular tissue
2	Mild infiltration
3	Moderate infiltration with moderate edema
4	Marked infiltration with marked edema
5	Severe infiltration with severe edema

Ankle Pannus

0	Normal
0.5	Minimal infiltration of pannus in cartilage and subchondral bone, affects only marginal zones and affects only a few joints
1	Minimal infiltration of pannus in cartilage and subchondral bone, primarily affects marginal zones
2	Mild infiltration (<1/4 of tibia or tarsals at marginal zones)
3	Moderate infiltration (1/4 to 1/3 of tibia or small tarsals affected at marginal zones)
4	Marked infiltration (1/2 to 3/4 of tibia or tarsals affected at marginal zones)
5	Severe infiltration (>3/4 of tibia or tarsals affected at marginal zones, severe distortion of overall architecture)

Knee Pannus

0	Normal
0.5	Minimal infiltration of pannus in cartilage and subchondral bone, affects only marginal zones and affects only a few joints
1	Minimal infiltration of pannus in cartilage and subchondral bone, approximately 1–10% of cartilage surface or subchondral bone affected

2 Mild infiltration (extends over up to1/4 of surface or subchondral area of tibia or femur), approximately 11–25% of cartilage surface or subchondral bone affected

3 Moderate infiltration (extends over >1/4 but < 1/2 of surface or subchondral area of tibia or femur) approximately 26–50% of cartilage surface or subchondral bone affected

4 Marked infiltration (extends over 1/2 to 3/4 of tibial or femoral surface) approximately 51–75% of cartilage surface or subchondral bone affected

5 Severe infiltration approximately 76–100% of cartilage surface or subchondral bone affected

Ankle Cartilage Damage (emphasis on small tarsals)

0 Normal

0.5 Minimal decrease in T blue staining, affects only marginal zones and affects only a few joints

1 Minimal = minimal to mild loss of toluidine blue staining with no obvious chondrocyte loss or collagen disruption

2 Mild = mild loss of toluidine blue staining with focal mild (superficial) chondrocyte loss and/or collagen disruption

3 Moderate = moderate loss of toluidine blue staining with multifocal moderate (depth to middle zone) chondrocyte loss and/or collagen disruption, smaller tarsals affected to 1/2 to 3/4 depth with rare areas of full thickness loss

4 Marked = marked loss of toluidine blue staining with multifocal marked (depth to deep zone) chondrocyte loss and/or collagen disruption, 1 or 2 small tarsals surfaces have full thickness loss of cartilage

5 Severe = severe diffuse loss of toluidine blue staining with multifocal severe (depth to tide mark) chondrocyte loss and/or collagen disruption affecting more than 2 cartilage surfaces

Knee Cartilage Damage

0 Normal

0.5 Minimal decrease in T blue staining, affects only marginal zones

1 Minimal = minimal to mild loss of toluidine blue staining with no obvious chondrocyte loss or collagen disruption

2 Mild = mild loss of toluidine blue staining with focal mild (superficial) chondrocyte loss and/or collagen disruption, may have few small areas of 50% depth of cartilage affected

3 Moderate = moderate loss of toluidine blue staining with multifocal to diffuse moderate (depth to middle zone) chondrocyte loss and/or collagen disruption, may have 1–2 small areas of full thickness loss affecting less than 1/4 of the total width of a surface and not more than 25% of the total width of all surfaces

4 Marked = marked loss of toluidine blue staining with multifocal to diffuse marked (depth to deep zone) chondrocyte loss and/or collagen disruption or 1 surface with near total loss and partial loss on others, total overall loss less than 50% of width of all surfaces combined

5 Severe = severe diffuse loss of toluidine blue staining with multifocal severe (depth to tide mark) chondrocyte loss and/or collagen disruption on both femurs and/or tibias, total overall loss greater than 50% of width of all surfaces combined

Ankle Bone Resorption

0 Normal

0.5 Minimal resorption affects only marginal zones and affects only a few joints

1 Minimal = small areas of resorption, not readily apparent on low magnification, rare osteoclasts

2 Mild = more numerous areas of resorption, not readily apparent on low magnification, osteoclasts more numerous, <1/4 of tibia or tarsals at marginal zones resorbed

3 Moderate = obvious resorption of medullary trabecular and cortical bone without full thickness defects in cortex, loss of some medullary trabeculae, lesion apparent on low magnification, osteoclasts more numerous, 1/4 to 1/3 of tibia or tarsals affected at marginal zones

4 Marked = Full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts, 1/2 to 3/4 of tibia or tarsals affected at marginal zones

5 Severe = Full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts, >3/4 of tibia or tarsals affected at marginal zones, severe distortion of overall architecture

Knee Bone Resorption

0 Normal

0.5 Minimal resorption affects only marginal zones

1 Minimal = small areas of resorption, not readily apparent on low magnification, approximately 1–10% of total joint width of subchondral bone affected

2 Mild = more numerous areas of resorption, definite loss of subchondral bone, approximately 11–25% of total joint width of subchondral bone affected

3 Moderate = obvious resorption of subchondral bone approximately 26–50% of total joint width of subchondral bone affected

4 Marked = obvious resorption of subchondral bone approximately 51–75% of total joint width of subchondral bone affected

5 Severe = distortion of entire joint due to destruction approximately 76–100% of total joint width of subchondral bone affected

Results:

Disease severity in the disease control group increased from days 1 to 5 with day 4–5 having the greatest daily increase. Then the incremental increases were smaller to the peak at day 7. From that point forward, acute swelling generally decreased and calliper measures were decreased. The treatment groups followed this general pattern as well.

Body weight loss was observed in all disease groups whereas the normal control group had a weight increase. Body weight loss was significantly (25%, $p < 0.05$ by ANOVA) inhibited for rats treated with 5 mg/kg SEQ ID NO: 172 as compared to vehicle treated disease controls. When compared to disease controls using a Student's *t*-test, inhibition of body weight loss was also significant for rats treated with 1 mg/kg SEQ ID NO: 172 (21%, $p < 0.05$) or Dex (21%, $p < 0.05$). Results of treatment with SEQ ID NO: 172 were dose responsive for this parameter.

Daily ankle diameter measurements were significantly ($p < 0.05$ by 2-way RM ANOVA) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 ($p < 0.05$ days 4–12) or Dex ($p < 0.05$ d3–14) as compared to disease controls.

Ankle diameter AUC was significantly ($p < 0.05$ by ANOVA) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 (43% reduction), 1 mg/kg SEQ ID NO: 172 (27%), or Dex (97%) as compared to disease controls. Results of treatment with SEQ ID NO: 172 were dose responsive for this parameter.

Final paw weights were significantly ($p < 0.05$ by ANOVA) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 (26% reduction) or Dex (114%) as compared to disease controls. Results of treatment with SEQ ID NO: 172 were dose responsive for this parameter.

Relative liver weights were not significantly (by ANOVA) affected for rats in any treatment group as compared to disease controls.

Spleen weights relative to body weight were significantly ($p < 0.05$ by ANOVA) reduced for rats treated with Dex as compared to disease controls. Relative spleen weights for Dex treated rats were also significantly reduced as compared to normal controls. Relative spleen weights were not significantly affected for rats treated with SEQ ID NO: 172.

Thymus weights relative to body weight were significantly ($p < 0.05$ by ANOVA) reduced for rats treated with Dex as compared to disease controls. Relative thymus weights for Dex treated rats were also significantly reduced as compared to normal controls. Relative thymus weights were not significantly affected for rats treated with SEQ ID NO: 172.

All ankle histopathology parameters were significantly (by Mann-Whitney U test) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 (25% reduction of summed scores) as compared to disease controls.

All knee histopathology parameters were significantly (by Mann-Whitney U test) reduced toward normal for rats treated with 5 mg/kg SEQ ID NO: 172 (73% reduction of summed scores) as compared to disease controls.

Results of this study indicated that daily intravenous treatment with SEQ ID NO: 172 (5 mg/kg) had significant beneficial effect on the clinical and histopathology parameters associated with established type II collagen arthritis in rats. Treatment with SEQ ID NO: 172 (1 mg/kg) resulted in significantly reduced ankle diameter AUC. The beneficial effect on ankle diameter was observed up to day 12 despite the reduction of swelling after day 7 in disease control animals. Results of treatment with SEQ ID NO: 172 were dose responsive.

Treatment with SEQ ID NO: 172 had no adverse effect on organ weights unlike dexamethasone.

Example 12: Effect of the all-D-retro-inverso JNK-inhibitor (poly-)peptide of SEQ ID NO: 197 and the JNK inhibitor (poly-)peptide of SEQ ID NO: 172 at three doses in a Scopolamine-Induced Model of Dry Eye in Mice

Study concept

The objective of this study was to assess the effects of two different compounds, the all-D-retro-inverso JNK-inhibitor (poly-)peptide of SEQ ID NO: 197 and the JNK inhibitor (poly-)peptide of SEQ ID NO: 172, at three dose levels in a mouse model of scopolamine-induced dry eye.

The peptides of SEQ ID NO: 197 and SEQ ID NO: 172 were tested for efficacy in this murine model of dry eye. The peptides were both tested at a low, medium and a high dose. For the peptide of SEQ ID NO: 197 the concentrations measured in the formulation samples for low, medium and high dose levels were 0.06% (w/v), 0.25% (w/v) and 0.6% (w/v), respectively, and for SEQ ID NO: 172 the concentrations measured in the formulation samples for the low, medium and high dose levels, were 0.05% (w/v), 0.2% (w/v) and 0.6% (w/v), respectively. The vehicle, which also served as the negative control, was 0.9% Sodium Chloride for Injection USP.

The study consisted of a total of 9 groups of female C57BL/6 mice, comprising 8 groups of 12 mice each and an additional group of 4 mice. Bilateral short-term dry eye was induced by a combination of scopolamine hydrobromide (Sigma-Aldrich Corp., St. Louis, MO) injection (subcutaneous (SC), four times daily, 0.5 mg/dose, Days 0-21) and by exposing mice to the drying environment of constant air draft. Starting on Day 1, mice of Groups 1–8 were treated three times daily (TID) for 21 days with bilateral topical ocular (oculus uterque; OU) administration (5 µL/eye/dose) of vehicle (0.9% sterile saline; negative control article); the peptide of SEQ ID NO: 197 (0.06%, 0.25% and 0.6%), the peptide of SEQ ID NO: 172 (0.05%, 0.2% and 0.6%); or cyclosporine (0.05%; positive control, an immunosuppressant drug used to reduce the activity of the immune system). Mice of Group 9 were maintained as un-induced, (no dry eye) untreated controls.

During the in-life (treatment) period, clinical observations were recorded once daily; slit-lamp examination (SLE) with corneal fluorescein staining, tear break-up time test (TBUT), and phenol red thread test (PRTT) were performed three times per week. Necropsies were performed on Day 22; eyes, eye lids, conjunctivae, and lacrimal glands were collected from both eyes of each animal. Tissues from the right eyes (oculus dexter, OD) were fixed and then evaluated microscopically. Tissues from the left eyes (oculus sinister; OS) were flash-frozen in liquid nitrogen and stored frozen at -80 °C for possible subsequent analyses.

Table 5: Experimental Design

Group	Number of animals (females)	Induction of Dry Eye (QID, SC) Days 0 to 21	Treatment (TID, OU, 5 µL/eye) Days 1 to 21
1	12	Scopolamine (200 µL of 2.5 mg/mL sol., 0.5 mg/dose)	Vehicle
2	12		SEQ ID NO: 197 (0.06%)
3	12		SEQ ID NO: 197 (0.25%)
4	12		SEQ ID NO: 197 (0.6%)
5	12		SEQ ID NO: 172 (0.05%)
6	12		SEQ ID NO: 172 (0.2%)
7	12		SEQ ID NO: 172 (0.6%)
8	12		Restasis®* (0.05%)
9	4	No dry eye induction	No treatment

* Cyclosporine

Methods

1. Dose preparation

The (poly-)peptide of SEQ ID NO: 197 was obtained from Polypeptide Laboratories (France) as a 1.5-mL clear plastic microfuge vial containing 300.65 mg of dry powder.

The (poly-)peptide of SEQ ID NO: 172 was obtained from Polypeptide Laboratories (France) as a 1.5-mL clear plastic microfuge vial containing 302.7 mg of dry powder.

Prior to the start of the study, the (poly-)peptides of SEQ ID NO: 172 and of SEQ ID NO: 197 were formulated in sterile saline (vehicle). Dosing solutions at each concentration were sterilized using 0.2-µm filters, aliquoted to multiple pre-labeled vials, and frozen at -20 °C. The concentrations measured in the formulation samples for the peptide of SEQ ID NO: 197 were 0.058%, 0.25% and 0.624%, rounded to 0.06%, 0.25% and 0.6%. The concentrations measured in the formulation samples for the peptide of SEQ ID NO: 172 were 0.053%, 0.217% and 0.562%, rounded to 0.05, 0.2% and 0.6%.

On each day of dosing, one set of dosing solutions was thawed and used for that day's dose administrations. The controls (vehicle, cyclosporine) were provided ready to dose; no dose preparation was necessary.

2. Slit-Lamp Examinations (SLE)

Prior to entry into the study, each animal underwent a SLE and indirect ophthalmic examination using topically-applied fluorescein. Ocular findings were recorded using the Draize scale ocular scoring. SLE and Draize scoring were repeated three times a week during the in-life period.

3. Tear Break-Up Time (TBUT) Test and Subsequent Corneal Examination

The TBUT test was conducted three times weekly by measuring the time elapsed in seconds between a complete blink after application of fluorescein to the cornea and the appearance of the first random dry spot in the tear film. To perform the TBUT, 0.1% liquid sodium fluorescein was dropped into the conjunctival sac, the eyelids were manually closed three times and then held open revealing a continuous fluorescein-containing tear film covering the cornea, and the time (in seconds) required for the film to break (appearance of a dry spot or streak) was recorded. At least ninety seconds later, corneal epithelial damage was graded using a slit-lamp with a cobalt blue filter after another drop of 0.1% fluorescein was reapplied to the cornea; the cornea then was scored per the Draize ocular scale.

4. Phenol Red Thread Tear Test (PRTT)

Tear production was measured three times a week in both eyes using PRTT test strips (Zone-Quick; Menicon, Nagoya, Japan). Prior to the first treatment of the day, a thread was applied to the lateral canthus of the conjunctival fornix of each eye for 30 seconds under slit-lamp biomicroscopy. Tear migration up the tread (i.e., the length of the wetted cotton thread) was measured using a millimeter scale.

5. Necropsy and Pathology

At necropsy on Day 22, both eyes from each animal, including the globes, lacrimal glands, eyelids, and conjunctivae, were excised. The right eye and associated tissues were fixed by overnight submersion in modified Davidson's solution followed by transfer to 10% neutral buffered formalin (NBF). The fixed tissues of the right eye were dehydrated, embedded in paraffin, sectioned at 3 to 5-μm thicknesses, and slide-mounted tissues were stained with hematoxylin and eosin (H & E). Stained slides were evaluated via light microscopy. Detailed and complete histopathologic assessment was conducted on all parts of the eye, with at least two section levels being examined histopathologically for each right eye. Special attention was paid to the cornea, epithelia (including goblet cells) of the conjunctiva and cornea, as well as the lacrimal gland. These tissues were scored for injury based upon a 0–4 scale, with 0 being normal, 1 being minimal, 2 being mild, 3 being moderate, and 4 being severe. For each cornea, scores were based

on corneal epithelium thickness, and corneal inflammation. Conjunctivae were scored for erosion and inflammation as well as presence or absence of goblet cells.

RESULTS

Four-times daily SC administration of scopolamine (0.5mg/dose) induced a dry eye syndrome in female C57BL/6 mice characterized by a decrease in the volume of aqueous tear production and changes in the physiochemical properties of the tears rendering them less capable of maintaining a stable tear film able to effectively lubricate and protect the eye.

1. Tear Break-Up Time (TBUT) Teat and Corneal Examination

The tear break-up time tests (TBUTs) were performed prior to the induction of dry eye, and again on Days 2, 4, 7, 9, 11, 14, 16, 18 and 21 after dry eye induction. After initiation of dosing with scopolamine (dry eye induction) TBUT mean values began to decrease in all animals, but appeared to decrease more slowly in Group 6 (mid-dose of SEQ ID NO: 172). The TBUT mean nadir for Groups 5, 6, 7 (low, mid and high-dose of the peptide of SEQ ID NO: 172), and Group 8 (cyclosporine) occurred on Day 7, reaching similar values (6.6 ± 0.4, 6.7 ± 0.4, 6.7 ± 0.3, and 6.4 ± 0.4 s, respectively). Subsequently, the TBUT means of these groups increased to a peak on Day 9. Groups 6 and 7 (SEQ ID NO: 172 mid and high-dose groups) TBUT means rose to higher values (10.0 ± 0.7 s and 9.9 ± 0.8 s, respectively) than Group 8, the cyclosporine group (8.5 ± 0.3 s), while the peak TBUT mean of Group 5, the low-dose of SEQ ID NO: 172 (8.0 ± 0.4 s) was slightly below that of Group 8 (cyclosporine). TBUT means for the mid and high-dose of SEQ ID NO: 197-treated animals, Groups 3 and 4, continued to decline after onset of dosing, reaching a nadir on Day 9, while the low-dose Group 2 increased on Day 9. The low, medium and high-dose TBUT means of SEQ ID NO: 172-treated animals (Groups 2, 3 and 4, respectively) were above the vehicle group and generally below the low, mid and high-dose group means of SEQ ID NO: 172-treated animals.

When the area under the curve (AUC) for TBUT values from Day 7 to Day 21 was used to compare the various treatments with the vehicle control, treatment with mid, low and high-dose of the peptide of SEQ ID NO: 172 (0.05%, 0.2% and 0.6%, respectively), Groups 5, 6, and 7, as well as animals treated with cyclosporine (0.05%), Group 8, showed significant increases in the TBUT AUC (Kruskal-Wallis nonparametric ANOVA). The peptide of SEQ ID NO: 172 appeared to produce a dose-dependent increase in TBUT, with the mid and high-doses often producing similar effects. Furthermore, there were no significant differences in TBUT AUC between the cyclosporine-treated group, the groups treated with three dose levels of SEQ ID NO: 172 and the un-induced group (Groups 5, 6, 7, 8, and 9). This finding suggests that all three doses of the

peptide of SEQ ID NO: 172 and cyclosporine were approximately equally effective in improving or reversing the ophthalmological changes that underlie the TBUT changes in this dry eye model.

Groups treated with low, mid and high dose levels of the peptide of SEQ ID NO: 197 (Groups 2-4) showed slight generally dose-dependent increases in TBUT which started to increase approximately two days later than animals treated with SEQ ID NO: 172 or cyclosporine.

Table 6: Mean Calculated TBUT AUC Values:

Group	TBUT AUC
Group 1	71.19
Group 2	88.54
Group 3	91.19
Group 4	89.98
Group 5	102.98
Group 6	119.08
Group 7	119.31
Group 8	116.1
Group 9	124.54

2. Phenol Red Thread Tear Test (PRTT)

PRTT tests were performed prior to the induction of dry eye, and again on Days 2, 4, 7, 9, 11, 14, 16, 18 and 21. PRTT values from Day 0 to Day 4 decreased in all mice that had dry eye induced, indicating a decrease in tear production after the administration of scopolamine and exposure to a drying environment of increased air draft created by the blowers. The nadir in PRTT in most groups occurred at approximately Day 7. PRTT kept decreasing in the vehicle control group (Group 1) reaching a nadir on Day 14. After the nadir, there was an increase in all dry eye groups. These findings indicate that initiation of scopolamine treatment one day earlier than initiation of compound treatment was sufficient to initiate physiological changes in the eye associated with dry eye syndrome. Even the cyclosporine–treated group showed a decrease in PRTT similar to other groups through approximately Day 7, then increased to a peak on Days 11-14, followed by a slight decrease. In the last PRTT test (Day 21) cyclosporine (Group 8), and Groups 6 and 7 all had similar PRTT values suggesting that both the mid and high-dose of the peptide of SEQ ID NO: 172 treatments have therapeutic effects similar to cyclosporine in increasing the aqueous tear production in this murine dry eye model.

Animals treated with the low, mid or high-dose of the peptide of SEQ ID NO: 172 produced significantly more aqueous tears compared to vehicle-treated animals. Thus, similar to TBUT, the peptide of SEQ ID NO: 172 produced generally dose-related significant increases in the production of aqueous tears in this model.

Groups treated with low, mid and high dose levels of the peptide of SEQ ID NO: 197 (0.06%, 0.25% and 0.6%, Groups 2, 3 and 4, respectively) showed generally dose-dependent increases in PRTT.

Table 7: Mean PRTT AUC Values

Group	PRTT AUC
Group 1	35.02
Group 2	39.96
Group 3	42.79
Group 4	43.17
Group 5	44.38
Group 6	44.85
Group 7	46.10
Group 8	49.44
Group 9	113.63

3. Histopathology

In this study histologic changes were generally confined to the cornea. Findings in the cornea consisted of increased keratinization of the corneal epithelial surface, increased thickness of the corneal epithelium, increased cellularity of the corneal epithelium, mildly increased incidence of mitosis of the basal epithelial layer consistent with increased epithelial cell turnover. These findings are indicative of a physiologic adaptive response to corneal drying and corneal surface irritation. Surface ulceration, corneal stromal edema and inflammatory infiltrate into the cornea were not seen in this study. The eyes in Group 9, the untreated group (normal mice, no scopolamine treatment), were within normal limits. There was some minimal nonsuppurative inflammation of the eye lids scattered throughout all groups, but the conjunctiva, retina, lacrimal glands and other parts of the eye were within normal limits. Goblet cells appeared to be within limits in all groups. Goblet cells are a primary producer of mucin which helps the tears form a stronger more adhesive film.

Mild to moderate corneal changes were noted in all groups except the untreated normal eye group (Group 9) and were slightly more severe in Group 1, the vehicle-treated group and Group 2, the low dose of the peptide of SEQ ID NO: 197, in comparison to the other treatment groups. These findings were consistent with the positive beneficial effects of increased tear production on the cornea.

When histological scores of the various treatment groups were compared to the histological scores in the cyclosporine group to determine if any other treatments produced "similar score reductions" to cyclosporine, Groups 4, 6, and 7 were found to be not significantly different than the cyclosporine group scores. Thus, these three treatments, mid and high-dose of the peptide of SEQ ID NO: 172 and the high-dose of the peptide of SEQ ID NO: 197, were the most effective, after cyclosporine, in reducing/ameliorating the corneal changes associated with this murine dry eye model.

Claims

1. JNK inhibitor, selected from the group consisting of:

a) a JNK inhibitor, which comprises an inhibitory (poly-)peptide sequence according to the following general formula:

X1-X2-X3-R-X4-X5-X6-L-X7-L-X8 (SEQ ID NO: 1),

wherein X1 is an amino acid selected from amino acids R, P, Q and r,
wherein X2 is an amino acid selected from amino acids R, P, G and r,
wherein X3 is an amino acid selected from amino acids K, R, k and r,
wherein X4 is an amino acid selected from amino acids P and K,
wherein X5 is an amino acid selected from amino acids T, a, s, q, k or is absent,
wherein X6 is an amino acid selected from amino acids T, D and A,
wherein X7 is an amino acid selected from amino acids N, n, r and K; and
wherein X8 is an amino acid selected from F, f and w, and
wherein an amino acid residue given in capital letters indicates an L-amino acid, while an amino acid residue given in small letters indicates a D amino acid residue,

with the proviso that at least one of the amino acids selected from the group consisting of X1, X2, X3, X5, X7 and X8 is/are a D-amino acid(s), and

b) a JNK inhibitor which comprises an inhibitory (poly-)peptide sequence sharing at least at least 80% sequence identity with SEQ ID NO: 1 as defined in a),
with the proviso that with respect to SEQ ID NO: 1 such inhibitory (poly-)peptide sequence sharing sequence identity with SEQ ID NO: 1 maintains the L-arginine (R) residue of SEQ ID NO: 1 at position 4 and the two L-leucine (L) residues of SEQ ID NO: 1 at positions 8 and 10 and that at least one of the remaining amino acids in said sequence sharing at least at least 80% sequence identity with SEQ ID NO: 1 is a D-amino acid,

for use in a method of treatment of the human or animal body by therapy.

2. JNK inhibitor for use according to claim 1, wherein at least one of the amino acids selected from the group consisting of X3, X5, X7 and X8 is/are a D-amino acid(s).

3. JNK inhibitor for use according to claim 1 or 2, wherein the JNK inhibitor comprises an inhibitory (poly-)peptide sequence sharing at least 80% sequence identity with a sequence selected from any one of SEQ ID NOs: 2-27.

4. JNK inhibitor for use according to anyone of the preceding claims, wherein the inhibitory (poly-)peptide sequence is selected from anyone of SEQ ID NOs: 2-27.

5. JNK inhibitor for use according to anyone of the preceding claims, wherein the JNK inhibitor comprises SEQ ID NO: 8 or an inhibitory (poly-)peptide sequence sharing at least 80% sequence identity with SEQ ID NO: 8.

6. JNK inhibitor for use according to anyone of the preceding claims, wherein the JNK inhibitor comprises a transporter sequence.

7. JNK inhibitor for use according to claim 6, wherein the inhibitory (poly-)peptide sequence and the transporter sequence overlap.

8. JNK inhibitor for use according to claim 6 or 7, wherein the transporter sequence comprises a sequence of alternating D- and L-amino acids according to anyone of SEQ ID NOs: 28-30.

9. JNK inhibitor for use according to anyone of claims 6-8, wherein said transporter sequence is selected from any one of SEQ ID NOs: 31-170.

10. JNK inhibitor for use according to anyone of claims 6-9, wherein said transporter sequence is selected from any one of SEQ ID NOs: 31-34, 46, 47 and 52-151.

11. JNK inhibitor for use according to anyone of claims 6-10, wherein said transporter sequence is positioned directly N-terminal or directly C-terminal of the inhibitory (poly-)peptide sequence.

12. JNK inhibitor for use according to anyone of claims 6-11, wherein the JNK inhibitor comprises

    a) a sequence according to any one of SEQ ID NOs: 171-190, or

b) a sequence sharing at least 50% sequence identity with at least one of SEQ ID NOs: 171-190, with the proviso that said sequence sharing sequence identity anyone of SEQ ID NOs: 171-190:

i) maintains the L-arginine (R) residue on position 4 in its sequence stretch corresponding to SEQ ID NO: 1,

ii) maintains the two L-leucine (L) in its sequence stretch corresponding to SEQ ID NO: 1, and

iii) exhibits at least one D-amino acid at positions X1, X2, X3, X5, X7 or X8 in its sequence stretch corresponding to SEQ ID NO: 1.

13. JNK inhibitor for use according to anyone of claims 6-12, wherein the JNK inhibitor comprises

a) the sequence of SEQ ID NO: 172 or

b) a sequence sharing 50% sequence identity with SEQ ID NO: 172, with the proviso that said sequence sharing 50% sequence identity with SEQ ID NO: 172

i) maintains the L-arginine (R) residue on position 4 in its sequence stretch corresponding to SEQ ID NO: 1,

ii) maintains the two L-leucine (L) in its sequence stretch corresponding to SEQ ID NO: 1, and

iii) exhibits at least one D-amino acid at positions X1, X2, X3, X5, X7 or X8 in its sequence stretch corresponding to SEQ ID NO: 1.

14. JNK inhibitor comprising:

a) an inhibitory (poly-)peptide comprising a sequence selected from the group of sequences consisting of RPTTLNLF (SEQ ID NO: 191), KRPTTLNLF (SEQ ID NO: 192), RRPTTLNLF and/or RPKRPTTLNLF (SEQ ID NO: 193), and

b) a transporter sequence selected from SEQ ID NOs: 31-34 and 46-151,

for use in a method for treatment of the human or animal body by therapy.

15. JNK inhibitor comprising the sequence of SEQ ID NO: 194 or 195 for use in a method for treatment of the human or animal body by therapy.

16. JNK inhibitor for use according to any one of claims 1 to 61, wherein said method is for treatment of the human body by therapy.

17. JNK inhibitor for use according to any one of claims 1 to 61, wherein said JNK inhibitor is administered intravenously, intramuscularly, subcutaneously, intradermally, transdermally, enterally, orally, rectally, topically, nasally, locally, intranasally, epidermally, by patch delivery, by instillation, intravitreally, subconjunctivally and/or intratympanically.

18. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from inflammatory diseases, diseases of the eye, diseases of the bone, neural diseases, neuronal diseases, neurodegenerative diseases, diseases of the skin, immune and/or autoimmune diseases, diseases of the eye, diseases of the mouth, metabolic diseases, cardiovascular diseases, proliferative diseases, diseases of the ear, diseases of the intestine, and/or diseases of the respiratory system.

19. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from inflammatory diseases, acute inflammation, chronic inflammation, inflammation in the eye, inflammation in the mouth, inflammation of the respiratory system, inflammation of the lung, inflammation of the skin, inflammation within the cardiovascular system, inflammation of the brain, inflammation in the ear, mucositis, stomatitis, peri-implantitis, retinitis, chorioiditis, keratoconjunctivitis sicca, inflammatory bowel diseases (IBD), uveitis, anterior uveitis, intermediate uveitis, posterior uveitis, periodontitis, COPD, asthma, pulpitis, rheumatoid arthritis, osteoarthritis, Crohn's disease, psoriatic arthritis, vasculitis, interstitial cystitis; acute inflammation at a site of infection or wound, meningitis, encephalitis, pneumonia, pharyngitis, tonsillitis, otitis, otitis media, vasculitis, synovitis, enteritis, Crohn's disease, ulcerative colitis and graft rejection.

20. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from ear diseases, diseases of the inner ear, hearing loss, acute hearing loss, damaged hair cell stereocilia, hair cell apoptosis, noise trauma, otitis and otitis media.

21. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from metabolic disorders, diabetes, diabetes type 1, diabetes type 2, Fabry disease, Gaucher disease, hypothermia, hyperthermia, hypoxia, lipid histiocytosis, lipidoses, metachromatic leukodystrophy, mucopolysaccharidosis, Niemann Pick disease, obesity, and Wolman's disease.

22. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from Alexander disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), apoplexy, Ataxia Telangiectasia, cut or otherwise disrupted axons, axotomy, brain lesions, CMT (Charcot-Marie-Tooth), corticobasal degeneration, dementia, diseases or disorders of the nervous system, dystonia, epilepsy, Farber's disease, Friedreich ataxia (SCA), gangliosidoses, Guillain-Barré syndrome, hereditary spastic paraplegia, Hirschsprung's disease, human immunodeficiency virus dementia, Huntington's disease, infarct of the brain, ischemic stroke, Krabbe disease, Lennox Gastaut Syndrome, lissencephaly, multiple sclerosis, myelodysplastic syndromes, myelopathy, AIDS-related neurodegenerative diseases, neurofibromatosis type 2 (NF-2), neurolatyerism, neuronal apoptosis, neuronal death, neuropathic pain, neuropathy, chemotherapy induced neuropathy, diabetes induced neuropathy, NMDA-induced neurotoxicity, pain, Parkinson's disease, parkinsonism, Pick's Disease, polyneuropathy, progressive supranuclear palsy, Sandhoff disease, spina bifida, stroke, Tay Sachs, TBI (diffuse axonal injury), treatment of dark neurone induced for example by an inflammatory pain, West Syndrome and spinal muscular atrophy.

23. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from autoimmune diseases of the CNS, auto-inflammatory diseases, Celiac disease; Sjogren's syndrome and systemic lupus erythematosus.

24. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from arthritis, disc herniation, fibrodysplasia ossificans progressiva (FOP), osteoarthritis, osteopetrosis, osteoporosis, diabetes induced osteoporosis, Paget's Disease and rheumatoid arthritis.

25. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from psoriasis and lupus erythematosus.

26. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from age-related macular degeneration (AMD); angioid streaks; anterior ischemic optic neuropathy; anterior uveitis; cataract, in particular age related cataract; central exudative chorioretinopathy; central serous chorioretinopathy; chalazion; chorioderemia; chorioiditis; choroidal sclerosis; conjunctivitis; cyclitis; diabetic retinopathy; dry eye syndrome; endophthalmitis;

episcleritis; eye infection; fundus albipunctatus; gyrate atrophy of choroid and retina; hordeolum; inflammatory diseases of the blephara; inflammatory diseases of the choroid; inflammatory diseases of the ciliary body; inflammatory diseases of the conjunctiva; inflammatory diseases of the cornea; inflammatory diseases of the iris; inflammatory diseases of the lacrimal gland; inflammatory diseases of the orbital bone; inflammatory diseases of the sclera; inflammatory diseases of the vitreous body; inflammatory diseases of the uvea; inflammatory diseases of the retina; intermediate uveitis; irititis; keratitis; Leber's disease; multifocal choroiditis; myositis of the eye muscle; neovascular maculopathy (e.g. caused by high myopia, tilted disc syndrome, choroidal osteoma or the like); NMDA induced retinotoxicity; non-chronic or chronic inflammatory eye diseases; Oguchi's disease; optic nerve disease; orbital phlegmon; panophtalmitis; panuveitis; post caspule opacification; posterior capsule opacification (PCO) (a cataract after-surgery complication); posterior uveitis; proliferative vitreoretinopathy; retinal artery occlusion; retinal detachment, retinal diseases; retinal injuries; retinal macroaneurysm; retinal pigment epithelium detachment; retinal vein occlusion; retinitis; retinitis pigmentosa; retinitis punctata albescens; retinopathy, in particular retinopathy of prematurity and diabetic retinopathy; scleritis; Stargardt's disease; treatment of inflamed ocular wounds and/or ocular wound edges; treatment of intraocular inflammation after eye surgery or trauma; uveitis; and vitelliform macular dystrophy.

27. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from periodontitis, in particular chronic periodontitis; mucositis, oral desquamative disorders, oral liquen planus, pemphigus vulgaris, peri-implantitis, pulpitis; stomatitis; and temporomandibular joint disorder.

28. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from cancer and tumor diseases, acusticus neurinoma lung carcinomas; acute lymphocytic leukemia (L1, L2, L3); acute lymphoid leukaemia (ALL); acute myelogenous leukemia (AML); adenocarcinomas; anal carcinoma; bronchial carcinoma; cervix carcinoma; cervical cancer; astrocytoma; basalioma; cancer with Bcr-Abl transformation; bladder cancer; blastomas; bone cancer; brain metastases; brain tumours; breast cancer; Burkitt's lymphoma; carcinoids; cervical cancer; chronic lymphocytic leukaemia (CLL); chronic myeloid leukaemia (CML); colon cancer; colon carcinoma; corpus carcinoma; craniopharyngeomas; CUP syndrome; virus-induced tumours; EBV-induced B cell lymphoma; endometrium carcinoma; erytholeukemia (M6); esophagus cancer; gallbladder cancer; gastrointestinal cancer; gastrointestinal stromal

tumors; gastrointestinal tumours; genitourinary cancer; glaucoma; glioblastoma; gliomas; head/neck tumours; hepatitis B-induced tumours; hepatocell carcinomas; hepatomas; herpes virus-induced tumours; Hodgkin's syndrome; HTLV-1-induced lymphomas; HTLV-2-induced lymphomas; insulinomas; intestinal cancer; Kaposi's sarcoma; kidney cancer; kidney carcinomas; laryngeal cancer; leukemia; lid tumour; liver cancer; liver metastases; lung cancer; lymphoid cancer; lymphomas; malignant melanomas; mammary carcinomas; mantle cell lymphoma; medulloblastoma; megakaryoblastic leukemia (M7); melanoma, in particular malignant melanoma; meningioma; mesothelioma; monocytic leukemia (M5); multiple myeloma; mycosis fungoides; myeloblastic leukemia (M1); myeloblastic leukemia (M2); myelomonocytic leukemia (M4); neurinoma; non-Hodgkin's lymphomas; non-small cell carcinoma; non-small cell carcinoma of the lung; oesophageal cancer; oesophageal carcinoma; oligodendroglioma; ovarian cancer; ovarian carcinoma; pancreatic cancer; pancreatic carcinoma; papilloma virus-induced carcinomas; penis cancer; pituitary tumour; plasmocytoma; promyelocytic leukemia (M3); prostate cancer; prostate tumours; rectal tumours; rectum carcinoma; renal-cell carcinoma; retinoblastoma; sarcomas; Schneeberger's disease; small cell lung carcinomas; small intestine cancer; small intestine tumours; soft tissue tumours; spinalioma; squamous cell carcinoma; stomach cancer; testicular cancer; throat cancer; thymoma; thyroid cancer; thyroid carcinoma; tongue cancer; undifferentiated AML (M0); urethral cancer; uterine cancer; vaginal cancer; Von Hippel Lindau disease; vulval cancer; Wilms' Tumor; and Xeroderma pigmentosum.

29. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from arterial hypertension; arteriosclerosis; arteriosclerotic lesions; Behcet's syndrome; bifurcations of blood vessels; cardiac hypertrophy; cardiavascular hypertrophy; cardiomyopathies, in particular chemotherapy induced cardiomyopathies; cerebral ischemia; coronary heart diseases; dilatation of the abdominal aorta; focal cerebral ischemia; global cerebral ischemia; heart hypertrophy; infrarenal aneurism hypertension; ischemia; myocardial infarct, in particular acute myocardial infarction; myocarditis; reperfusion; restenosis; vasculitis; and Wegener's granulomatosis.

30. JNK inhibitor for use according to any one of claims 1 to 17, wherein the JNK inhibitor is used complementary to coronary artery bypass graft surgery (CABG surgery); percutaneous transluminal coronary angioplasty (PTCA); and/or stent treatment.

31. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from acute respiratory distress syndrome (ARDS); asthma; chronic illnesses involving the respiratory system; chronic obstructive pulmonary disease (COPD); cystic fibrosis; diseases of the lung; inflammatory lung diseases; pneumonia; pulmonary fibrosis; etc.

32. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from colitis, atypical colitis, chemical colitis; collagenous colitis, distal colitis, diversion colitis; fulminant colitis, indeterminate colitis, infectious colitis, ischemic colitis, lymphocytic colitis, microscopic colitis, Crohn's disease, gastroenteritis, Hirschsprung's disease, inflammatory digestive diseases; inflammatory bowel disease (IBD), Morbus Crohn, non-chronic or chronic digestive diseases, non-chronic or chronic inflammatory digestive diseases; regional enteritis and ulcerative colitis.

33. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from bacterial infectious diseases, viral infectious diseases, viral encephalitis; viral induced cancers and tumours, human immunodeficiency virus dementia, meningitis, meningoencephalitis, encephalomyelitis and tonsillitis.

34. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diseases and/or disorders selected from Aarskog syndrome, acetaminophen hepatotoxicity; Alder-Reilly anomaly; alopecia areata; alpha-1-antitrypsin deficiency; anaphylaxis; apoptosis; apoptotic cell death; atypical hemolytic uremic syndrome; basopenia; basophilia; bipolar disorders; burns; cellular shear stress; Chedial-Higashi syndrome; DNA damage due to chemotherapeutic drugs; cholestasis; chromosome 11, Partial Monosomy 11q; chromosome 22, Trisomy Mosaic; chronic granulomatous disease; hepatitis, such as chronic or fulminant hepatitis; clinical depression; common variable hypogammaglobulinemia; congenital C3 deficiency; CTL protection from activation-induced cell death (AICD); deafness; depression and depressive disorders (in particular prevention of depressive disorders develop on a background of cytokine-induced sickness behaviour), DiGeorge's syndrome; diseases caused by defective apoptosis; diseases of the liver; diseases of the spine; diseases of the uterus; diseases states and symptoms due to exposure to DNA damaging agents and/or ionizing radiation and resulting cellular stress; Down Syndrome; Duchenne muscular dystrophy; ectodermal dysplasias; endometriosis;

eosinopenia; eosinophilia; exocitoxic cell death; fetal alcohol syndrome; fibrosis; fibrotic disease; formation of fibrous tissue; free radicals (leading to cellular stress); graft rejection; graft versus host disease; hair loss; hemolytic uremic syndrome; hepatotoxicity; hyperalgesia, such as diabetes induced hyperalgesia; hyperthermia; hypoglycemia; hypothyroidism; idiopathic hypereosinophilic syndrome; IgA nephropathy; infantile sex-linked agammaglobulinemia; inflammatory pain; infrarenal aneyrism; islet regeneration; islet transplantation; Job's syndrome (hyper-IgE); lazy leukocyte syndrome; leukocyte glucose-6-phosphate dehydrogenase deficiency; leukodystrophy; leukopenia; lymphocytic leukocytosis; lymphocytopenia; lymphocytosis; major depression; mania; maniac depression; Marfan syndrome; mastocytosis; May Hegglin Anomaly; membranoproliferative glomerulonephritis Type II; monocytopenia; monocytosis; myeloperoxidase deficiency-benign; myopathies; neutropenia; neutrophilia; Nezelof's syndrome; organ transplantation; oxidative stress injuries; Pelger-Huet anomaly; polycystic kidney diseases; post-dialysis syndrome; radiation syndromes; radiotherapy; renal diseases; renal failure; rescuing CTL from activation induced cell death; severe combined immunodeficiency disease; transplant rejection; transplantation; trisomy; unipolar depression; UV-induced injuries; Wiskott Aldrich syndrome and wound healing.

35. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of temporomandibular joint disorder.

36. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of mucositis.

37. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of stomatitis.

38. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of peri-implantitis.

39. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of oral liquen planus.

40. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of Pemphigus vulgaris.

41. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of periodontitis.

42. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of chronic periodontitis.

43. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of pulpitis.

44. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of uveitis, in particular of anterior uveitis, intermediate uveitis and/or posterior uveitis.

45. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of dry eye syndrome.

46. JNK inhibitor for use according to any one of claims 1 to 17, wherein the JNK inhibitor is administered in context of coronary artery bypass graft surgery.

47. JNK inhibitor for use according to any one of claims 1 to 17, wherein the JNK inhibitor is administered in context of percutaneous transluminal coronary angioplasty (PTCA).

48. JNK inhibitor for use according to any one of claims 1 to 17, wherein the JNK inhibitor is administered in context of stent placement.

49. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of acute myocardial infarction.

50. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of atherosclerosis.

51. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of COPD.

52. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of asthma.

53. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of rheumatoid arthritis.

54. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of osteoarthritis.

55. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of Crohn's disease.

56. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of inflammatory bowel disease (IBD).

57. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of psoriasis.

58. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of diabetes, in particular diabetes type 1.

59. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of stroke.

60. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of Parkinson's disease.

61. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of Alzheimer's disease.

62. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of systemic lupus erythematosus.

63. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of vasculitis, in particular Wegener's granulomatosis.

64. JNK inhibitor for use according to any one of claims 1 to 17, wherein said method is for treatment of acute hearing loss.

65. JNK inhibitor for use according to any one of the preceding claims, wherein the JNK inhibitor consists of the sequence of SEQ ID NO: 172.

66. Pharmaceutical composition comprising a JNK inhibitor as defined in any of claims 1 to 15 and a pharmaceutically acceptable carrier.