(54) Title: TREATMENT OF MIGRAINE AND HEADACHES WITH SELECTIVE PACl INHIBITORS

(57) Abstract: The present invention relates to the use of selective PACl inhibitors in the manufacture of a medicament for the treatment of migraine or headache.
Treatment of migraine and headaches with selective PAC1 inhibitors

All patent and non-patent references cited in the present application, are also hereby incorporated by reference in their entirety.

Field of invention

The present invention relates to the use of selective PAC1 inhibitors in the manufacture of a medicament for the treatment of migraine or headache.

Background of invention

Vasoactive intestinal peptide (VIP) and pituitary adenyl cyclase activating peptide (PACAP) are two closely related neuropeptides originally discovered in the lung and enteric nerves and in the hypothalamus, respectively. Structurally, they are part of a peptide superfamily which includes secretin, glucagon, growth hormone-releasing factor, and parathyroid hormone related peptide. Mature VIP occurs almost exclusively as a 28 amino acid amidated peptide, whereas PACAP exists primarily as a 38 amino acid peptide, but also as a 27 amino acid peptide truncated at the carboxy terminal. The human forms of VIP and PACAP are 68% identical. The peptides are widely, but differentially expressed in the central and peripheral nervous systems [1] as well as in endocrine and immune organs. These peptides classically act as neurotransmitters or neuromodulators, regulating diverse processes such as circadian rhythms, gastrointestinal, cardiovascular, and reproduction functions. More recently they have been implicated as growth and cell survival factors and as immuno-modulators. Three high affinity VIP and PACAP receptors that mediate the actions of these peptides have been identified to date. Each of them is a seven transmembrane heterotrimeric G protein-coupled receptor (GPCR). Two of these, VPAC1 and VPAC2, bind VIP and PACAP with equal high affinity, whereas a third, PAC1, classically binds only PACAP with high affinity. All of these receptors signal via Gs to stimulate adenyl cyclase, but coupling to other G proteins and second messenger systems has been demonstrated in certain cases. Alternate splicing leads to several isoforms of PAC1 in humans (up to ten in rodents), whereas two isoforms have been identified for VPAC2. These splice variants alter ligand affinity and to some extent selectivity, and in some cases confer
coupling to different signalling pathways. The receptors are widely distributed on various tissues throughout the body, and exist on nearly all major types of immune cells, including B and T lymphocytes, macrophages, neutrophils, and natural killer cells (Neuropeptide Mimetics and Antagonists in the Treatment of Inflammatory Disease: Focus on VIP and PACAP, by Catalina Abad, Rosa P. Gomariz and James A. Waschek, Current Topics in Medicinal Chemistry, 2006, 6, 151-163).

A migraine headache is a form of vascular headache. Migraine has been defined by the international headache society in its classification of headache disorders, 2nd edition (IHCD-2). Migraine in this application is defined according to IHCD-2 (Headache_Classification_Subcommittee_of_the_International_Headache_Society (2004). "The International Classification of Headache Disorders: 2nd edition." Cephalalgia 24 Suppl 1:9-160).

Migraine headache is caused by a combination of vasodilatation (enlargement of blood vessels) and the release of chemicals from nerve fibres that coil around the blood vessels. During a migraine attack, the temporal, dural and pial arteries enlarge. Enlargement of the arteries stretches the nerves that coil around the arteries and cause the nerves to release chemicals. Among these chemicals are calcitonin gene-related peptide and other peptides and monoamines. They cause inflammation, pain, and further enlargement of the arteries. The increasing enlargement of the arteries magnifies the pain.

Migraine attacks are commonly associated with nausea, vomiting, diarrhoea and delayed emptying of the stomach into the small intestine which prevents oral medications from entering the intestine and being absorbed. The impaired absorption of oral medications is a common reason for the ineffectiveness of medications taken to treat migraine headaches, to the attack is also associated with pallor of the skin as well as cold hands and feet and increased sensitivity to light and sound sensitivity as well as blurred vision.

In migraine, drug therapy can be used in two ways: to prevent the attack or to relieve symptoms after the headache occurs.
If a person suffers infrequently from migraines, drugs can be taken at the first sign of
a headache to stop or ease the pain.

If a person suffer frequently from migraines, both pain relief and prophylactic
measures may be used. For many years ergotamine was the only drug available to
address severe migraine pain relief. Now there are newer, more effective drugs
available - imitrex, Zomig, Maxalt, Amerg are some choices for relief of the pain of
migraine. For headaches that occur three or more times a month, preventive
treatment is often recommended. Drugs used to prevent migraine include betan
inhibitors, antiepileptics, NSAID's and amine antagonists e.g. methysergide, which
counteracts blood vessels by blocking the activity of serotonin at one type of
receptor while mimicking the effect of serotonin at another type of receptor.

The medicaments developed to date to prevent or stop migraine have different
effects on different persons, including different side-effects. Therefore there is a
continued need to develop new medicaments for treating migraine.

Summary of invention

The present invention relates to the use of selective PAC1 inhibitors in the
manufacture of a medicament for the treatment of migraine or other headaches. The
selective PAC1 inhibitors block PACAP from binding to PAC1, but in a preferred
embodiment the inhibitor does not block binding to VPAC1 and VPAC2.

It has been found that inhibition of PAC1 by selectively blocking PACAP from
binding to PAC1 receptor then migraine and/or other headaches may be treated.

Also disclosed are methods for identifying selective PAC1 inhibitors capable of
blocking binding of PACAP to PAC1.

Selective PAC1 inhibitors can be utilised to produce a pharmaceutical composition
comprising an effective amount of at least one selective PAC1 inhibitor further
information of the inhibitors are specified herein. For the pharmaceutical
composition also a salt of the at least one selective PAC1 inhibitor can be used,
onoptionally further comprising a pharmaceutically acceptable carrier substance.
Also disclosed is a method for treating or alleviating a disorder or disease of a living animal body, including a human, which disorder or disease is responsive to selective inhibition of PAC1, the method comprises administering to such a living animal body a therapeutically-effective amount of a selective PAC1 inhibitor as further described herein.

The compounds mentioned above can be used individually as the selective PAC1 inhibitor or two or more compounds can be incorporated into the medicament. The medicament may be formulated in any suitable manner as described below.

In one embodiment of the invention the selective PAC1 inhibitor is used for treatment of migraine, where said treatment is prophylactic and/or acute. The person skilled in the art knows the meaning of prophylactic and/or acute treatment, respectively, of migraine.

The selective PAC1 inhibitor may be administered as a systemic treatment or as a local treatment, for example in the form of a depot treatment.

**Detailed description of the invention**

In one aspect of the present invention at least one selective PAC1 inhibitor is used for the manufacture of a medicament for treatment or alleviation of migraine and/or other headaches. The ICHD-2 defines, in addition to migraine definitions to all other headaches. When this application refers to other headaches it means all headaches defined in IHCD-2 (Headache_Classification_Subcommittee_of_the_International_Headache_Society 2004). "The International Classification of Headache Disorders: 2nd edition." Cephalalgia 24 Suppl 1:9-1 60).

A selective PAC1 inhibitor is an inhibitor of the binding of PACAP specifically to PAC1. The selective PAC1 inhibitors used for the manufacture of the medicament can be any selective PAC1 inhibitor, and are not limited to the selective PAC1 inhibitors mentioned herein. Details of selective PAC1 inhibitors are listed herein below.
The sequence of PACAP (SEQ ID NO: 1) is shown below:

MTMCSGARLA LLVYGIIMHS SVYSSPAAG LRFPGIRPEE EAYGEDGNPL 50

PDGDGSEPPG AGSPASAPRA AAAWYPAGR RDOVHILNE AYRKVLQDLS 100

AGKHLQSLVA RGVGGSLLGG AGDDEPSLKR RHSQDFTDS YSRYKQMAV 150

KKYLAAVLGK RYKQRVKNKG RRHALY 176

The sequence includes signal peptide, propeptide, and peptides.

Two splice variants of PACAP are known, i.e. PACAP27 and PACAP38. PACAP27 corresponds to amino acid 132-158, and PACAP38 corresponds to amino acid 132-169 of the above sequence SEQ ID NO:1.

PAC1 is a receptor for PACAP, and PACAP38 and PACAP27 bind with high affinity to PAC1, 9.0-8.4 pKi, and 8.7-8.0 pKi, respectively. The amino acid sequence of PAC1 is found at SwissProt P41586. The length of unprocessed PAC1 is 468 amino acid, and the sequence is shown herein as SEQ ID NO: 2:

The first 20 amino acids correspond to the signal peptide. The remaining 448 amino acids constitute the cytoplasmic and extracellular parts, in that it appears that PAC1 is a multi-pass membrane protein, whereby several parts of the amino acid sequence are located as extracellular parts, and intervening parts are cytoplasmic parts.

Without being bound by theory, it is believed that the cytoplasmic parts and extracellular parts are arranged as follows (source SwissProt):
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</table>

Furthermore, according to literature PAC1 has several optional splice variants, such as 5 or 8 splice variants. In the following the possible splice variants described in the database GeneCards are shown.
5 Alternative splice patterns (SP)

5 Alternative splice patterns (SP)

8 Alternative splice patterns (SP)
In particular the inhibitor of the PAC1 according to the invention is an inhibitor of binding of PACAP to PAC1 or at least one of the isoforms of PAC1. In particular the inhibitor is an inhibitor of two or more of the isoforms of PAC1.

In preferred embodiment of the present invention the inhibitor has a higher affinity for PAC1 or for at least one isoform of PAC1 than the affinity of PACAP to PAC1 or said PAC1 isoform. The affinity of PACAP to PAC1 is as described above.

Preferably the affinity of the inhibitor to PAC1 is at least 1, 2, or 3 times higher than that of PACAP, such as equal to or above 5, such as equal to or above 10, such as equal to or above 15, such as equal to or above 20, such as equal to or above 25, such as equal to or above 30, such as equal to or above 35, such as equal to or above 40, such as equal to or above 45, such as equal to or above 50, such as equal to or above 55, such as equal to or above 60, such as equal to or above 65, such as equal to or above 70, such as equal to or above 75, such as equal to or above 80, such as equal to or above 85, such as equal to or above 90, such as equal to or above 95, such as equal to or above 100, such as equal to or above 105, such as equal to or above 110, such as equal to or above 115, such as equal to or above 120, such as equal to or above 125, such as equal to or above 150, such as equal to or above 175, such as equal to or above 200, such as equal to or above 225, such as equal to or above 250, such as equal to or above 275, such as equal to or above 300, such as equal to or above 325, such as equal to or above 350, such as equal to or above 375, such as equal to or above 400, such as equal to or
above 425, such as equal to or above 450, such as equal to or above 475, such as equal to or above 500 times higher than that of PACAP.

In the present context binding affinity is measured by as described in Example 1 below.

The affinity of the selective inhibitor for PAC1 is the same or higher than said inhibitors affinity for VPAC1 and/or VPAC2. Preferably the selective inhibitor has a higher affinity for PAC1 than said inhibitors affinity for VPAC1 and/or VPAC2.

It is preferred that the selective inhibitor does not block VPAC1 and/or VPAC2. In a preferred embodiment, in order to avoid side effects, the selective PAC1 inhibitor should in the doses given, not block VPAC1 and/or VPAC2. The selective inhibitor preferably does not block VPAC1. Likewise the selective inhibitor does not block VPAC2. More preferably, the selective inhibitor does not block any of VPAC1 or VPAC2.

Preferably the affinity of the inhibitor to PAC1 is at least 1, 2 or 3 times higher than the inhibitors affinity to VPAC1 and/or VPAC2, such as equal to or above 4, 5 or 6 times higher than the affinity to VPAC1 and/or VPAC2, such as equal to or above 7, 8 or 9 times higher than the affinity to VPAC1 and/or VPAC2, such as equal to or above 10 times higher than the inhibitors affinity to VPAC1 or VPAC2, such as equal to or above 15, such as equal to or above 20, such as equal to or above 25, such as equal to or above 30, such as equal to or above 35, such as equal to or above 40, such as equal to or above 45, such as equal to or above 50, such as equal to or above 55, such as equal to or above 60, such as equal to or above 65, such as equal to or above 70, such as equal to or above 75, such as equal to or above 80, such as equal to or above 85, such as equal to or above 90, such as equal to or above 95, such as equal to or above 100, such as equal to or above 105, such as equal to or above 110, such as equal to or above 115, such as equal to or above 120, such as equal to or above 125, such as equal to or above 150, such as equal to or above 175, such as equal to or above 200, such as equal to or above 225, such as equal to or above 250, such as equal to or above 275, such as equal to or above 300, such as equal to or above 325, such as equal to or above 350, such as equal to or above 375, such as equal to or above 400, such as equal to or above
425, such as equal to or above 450, such as equal to or above 475, such as equal to or above 500 times higher than the inhibitors affinity to VPAC1 or VPAC2.

The affinity to VPAC1 and VPAC2 is measured as described above for affinity to PAC1.

Furthermore, in order to be a selective inhibitor, the compound of the present invention must also function as an antagonist of PACAP38 or PACAP27, i.e. by blocking PAC1. The inhibition of PAC1 may be measured by a variety of methods, one of which is disclosed in Example 2.

Selective PAC1 inhibitors according to the present invention may be any type of compounds, such as peptides, antibodies as well as small organic chemical structures. In particular in relation to peptides and antibodies it is preferred that the length of the peptide or antibody is short, such as preferably below 20 amino acids, more preferably below 15 amino acids, and more preferably below 10 amino acids. Examples of selective inhibitors are:

Inhibitory peptides

Inhibitory fragments or variants of PACAP, such as PACAP6-38 or fragments thereof, wherein PACAP6-38 corresponds to amino acids 137-169 of SEQ ID NO: 1.

Inhibitory fragments or variants of the vasodilatory peptide Maxadilan, such as M65 disclosed in "Functional Characterization of Structural Alterations in the Sequence of the Vasodilatory Peptide Maxadilan Yields a Pituitary Adenylate Cyclase-activating Peptide Type 1 Receptor-specific Antagonist" By Moro et al, Vol. 274, No. 33, Issue of August 13, pp. 231 03-231 10, 1999.

In one aspect the term "variant of a peptide sequence" means that the peptides may be modified, for example by substitution of one or more of the amino acid residues. Both L-amino acids and D-amino acids may be used. Other modification may comprise derivatives such as esters, sugars, etc. Examples are methyl and acetyl esters.
In another aspect "variants" may be understood as exhibiting amino acid sequences gradually differing from the preferred predetermined sequence, as the number and scope of insertions, deletions and substitutions including conservative substitutions increase. This difference is measured as a reduction in homology between the predetermined sequence and the variant.

In still another aspect, variants of the peptide fragments according to the invention may comprise, within the same variant, or fragments thereof or among different variants, or fragments thereof, at least one substitution, such as a plurality of substitutions introduced independently of one another. Variants of the peptides, or fragments thereof may thus comprise conservative substitutions independently of one another, wherein at least one glycine (Gly) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and lie, and independently thereof, variants, or fragments thereof, wherein at least one alanine (Ala) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Val, Leu, and lie, and independently thereof, variants, or fragments thereof, wherein at least one valine (Val) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Leu, and lie, and independently thereof, variants, or fragments thereof, wherein at least one leucine (Leu) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and lie, and independently thereof, variants, or fragments thereof, wherein at least one isoleucine (Ile) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, variants, or fragments thereof wherein at least one aspartic acids (Asp) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Glu, Asn, and Gln, and independently thereof, variants, or fragments thereof, wherein at least one asparagine (Asn) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, variants, or fragments thereof, wherein at least one glutamine (Gln) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and wherein at least one phenylalanine (Phe) of said variants, or fragments thereof is...
substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, variants, or fragments thereof, wherein at least one tyrosine (Tyr) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, variants, or fragments thereof, wherein at least one arginine (Arg) of said fragment is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, variants, or fragments thereof, wherein at least one lysine (Lys) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, variants, or fragments thereof, and independently thereof, variants, or fragments thereof, and wherein at least one proline (Pro) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, variants, or fragments thereof, wherein at least one cysteine (Cys) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

It thus follows from the above that the same functional equivalent of a peptide fragment, or fragment of said functional equivalent may comprise more than one conservative amino acid substitution from more than one group of conservative amino acids as defined herein above. The term “conservative amino acid substitution” is used synonymously herein with the term “homologous amino acid substitution”.

The groups of conservative amino acids are as the following:
P, A, G (neutral, weakly hydrophobic),
S, T (neutral, hydrophilic)
Q, N (hydrophilic, acid amine)
E, D (hydrophilic, acidic)
H, K, R (hydrophilic, basic)
L, I, V, M, F, Y, W (hydrophobic, aromatic)
C (cross-link forming)
According to the invention, a variant may be an amino acid sequence having at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably 95%, even more preferably 97%, 98% or 99% homology to an amino acid sequence selected from SEQ ID NO:1 or the amino acid sequence of M65, or it may be an amino acid sequence having at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably 95%, even more preferably 97%, 98% or 99% positive amino acid matches compared to the amino acid sequence SEQ ID NO:1 or the amino acid sequence of M65. A positive amino acid match is defined herein as an identity or similarity defined by physical and/or chemical properties of the amino acids having the same position in two compared sequences. Preferred positive amino acid matches of the present invention are K to R, E to D, L to M, Q to E, I to V, I to L, A to S, Y to W, K to Q, S to T, N to S and Q to R. The homology of one amino acid sequence with another amino acid is defined as a percentage of identical amino acids in the two collated sequences. The wording "sequence homology" is used herein synonymously with the term "sequence similarity". The sequence homology, as already mentioned above, may be routinely calculated using well known algorithms such as BLOSUM 30, BLOSUM 40, BLOSUM 45, BLOSUM 50, BLOSUM 55, BLOSUM 60, BLOSUM 62, BLOSUM 65, BLOSUM 70, BLOSUM 75, BLOSUM 80, BLOSUM 85, or BLOSUM 90; Substitution of amino acids in a peptide sequence of the invention which results in formation of the peptide sequence variants included in the scope of the invention may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like. Exemplary amino acid substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine. In one embodiment the fragments described above are small peptides, such as dipeptides, or tripeptides, optionally being protected in one or both ends thereby reducing acid degradation of the peptides and/or increasing the half-life of the peptides. Such small peptides may for example be useful for oral administration.
Inhibitory antibodies or fragments thereof

In another embodiment the selective inhibitor is an inhibitory antibody or fragments thereof, wherein said antibody is raised against PAC1 or a fragment of PAC1 and capable of recognizing and selectively binding to an epitope comprising or comprised by an amino acid sequence selected from SEQ ID NO:2, or fragment, variant or homologue of said sequence. In a preferred embodiment the epitope comprising an amino acid sequence of the invention is located in extracellular part of PAC1.

By the term "epitope" is meant the specific group of atoms (on an antigen molecule) that is recognized by (that antigen's) antibodies (thereby causing an immune response). The term "epitope" is the equivalent to the term "antigenic determinant". The epitope may comprise 3 or more amino acid residues, such as for example 4, 5, 6, 7, 8 amino acid residues, located in close proximity, such as within a contiguous amino acid sequence, or located in distant parts of the amino acid sequence of an antigen, but due to protein folding have been approached to each other.

Antibody molecules belong to a family of plasma proteins called immunoglobulins, whose basic building block, the immunoglobulin fold or domain, is used in various forms in many molecules of the immune system and other biological recognition systems. A typical immunoglobulin has four polypeptide chains, containing an antigen binding region known as a variable region and a non-varying region known as the constant region.

Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the
heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains (Novotny J, & Haber E. Proc Natl Acad Sci U S A. 82(14):4592-6, 1985).

Depending on the amino acid sequences of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are at least five (5) major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG-1, IgG-2, IgG-3 and IgG-4; IgA-1 and IgA-2. The heavy chains constant domains that correspond to the different classes of immunoglobulins are called alpha (α), delta (δ), epsilon (ε), gamma (γ) and mu (μ), respectively. The light chains of antibodies can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino sequences of their constant domain. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "variable" in the context of variable domain of antibodies, refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies. The variable domains are for binding and determine the specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed through the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) also known as hypervariable regions both in the light chain and the heavy chain variable domains.

The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.
An antibody that is contemplated for use in the present invention thus can be in any of a variety of forms, including a whole immunoglobulin, an antibody fragment such as Fv, Fab, and similar fragments, a single chain antibody which includes the variable domain complementarity determining regions (CDR), and the like forms, all of which fall under the broad term "antibody", as used herein. The present invention contemplates the use of any specificity of an antibody, polyclonal or monoclonal, and is not limited to antibodies that recognize and immunoreact with a specific antigen. In preferred embodiments, in the context of both the therapeutic and screening methods described below, an antibody or fragment thereof is used that is immunospecific for an antigen or epitope of the invention.

The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', F(ab') \(_2\) and Fv fragments. Papain digestion of antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily. Pepsin treatment yields an F(ab') \(_2\) fragment that has two antigen binding fragments that are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and F(ab') \(_2\) fragments.

The term "antibody fragment" is used herein interchangeably with the term "antigen binding fragment".

Antibody fragments may be as small as about 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 9 amino acids, about 12 amino acids, about 15 amino acids, about 17 amino acids, about 18 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids or more. In general, an antibody fragment of the invention can have any upper size limit so long as it is has similar or immunological properties relative to antibody that binds with specificity to an epitope comprising a peptide sequence selected from any of the sequences identified herein as SEQ ID NOs: 1-39, or a fragment of said sequences. Thus, in context of the present
Antibody fragments retain some ability to selectively bind with its antigen or receptor. Some types of antibody fragments are defined as follows:

(1) Fab is the fragment that contains a monovalent antigen-binding fragment of an antibody molecule. A Fab fragment can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain.

(2) Fab' is the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per antibody molecule. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

(3) (Fab')2 is the fragment of an antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction.

(4) F(ab')2 is a dimer of two Fab' fragments held together by two disulfide bonds.

Fv is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (VH-VL dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Such single chain antibodies are also referred to as "single-chain Fv" or "sFv" antibody fragments. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the sFv to form the

The term "diabodies" refers to a small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161, and Hollinger et al., Proc. Natl. Acad Sci. USA 90: 6444-6448 (1993).

The invention contemplate both polyclonal and monoclonal antibody, antigen binding fragments and recombinant proteins thereof which are capable of binding an epitope according to the invention.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green et al. 1992. Production of Polyclonal Antisera, in: Immunochemical Protocols (Manson, ed.), pages 1-5 (Humana Press); Coligan, et al., Production of Polyclonal Antisera in Rabbits, Rats Mice and Hamsters, in: Current Protocols in Immunology, section 2.4.1, which are hereby incorporated by reference.

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, Nature, 256:495-7 (1975); Coligan, et al., sections 2.5.1-2.6.7; and Harlow, et al., in: Antibodies: A Laboratory Manual, page 726, Cold Spring Harbor Pub. (1988), Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan, et al., sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes, et al., Purification of Immunoglobulin G (IgG). In: Methods in Molecular Biology, 1992, 10:79-104, Humana Press, NY.
Methods of *in vitro* and *in vivo* manipulation of monoclonal antibodies are well known to those skilled in the art. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature 256, 495-7, or may be made by recombinant methods, e.g., as described in US 4,816,567. The monoclonal antibodies for use with the present invention may also be isolated from phage antibody libraries using the techniques described in Clackson et al., 1991, Nature 352: 624-628, as well as in Marks et al., 1991, J Mol Biol 222: 581-597. Another method involves humanizing a monoclonal antibody by recombinant means to generate antibodies containing human specific and recognizable sequences. See, for review, Holmes, et al., 1997, J Immunol 158:2192-2201 and Vaswani, et al., 1998, Annals Allergy, Asthma & Immunol 81:105-115.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the
desired biological activity (US 4,816,567); Morrison et al., 1984, Proc Natl Acad Sci 81:6851-6855.

Methods of making antibody fragments are also known in the art (see for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, NY, 1988, incorporated herein by reference). Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in E. coli of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')2. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, in US 4,036,945 and US 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent or the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as E. coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow, et al., 1991 , in: Methods: A Companion to Methods in
Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") are often involved in antigen recognition and binding. CDR peptides can be obtained by cloning or constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells.


The invention contemplates human and humanized forms of non-human (e.g. murine) antibodies. Such humanized antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain a minimal sequence derived from non-human immunoglobulin, such as the epitope recognising sequence. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a nonhuman species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. Humanized antibody(es) containing a minimal sequence(s) of antibody(es) of the invention, such as a sequence(s) recognising an epitope(s) described herein, is one of the preferred embodiments of the invention.

In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, humanized antibodies will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that

The generation of antibodies may be achieved by any standard methods in the art for producing polyclonal and monoclonal antibodies using natural or recombinant fragments of PAC1 which comprise an amino acid sequence as described above. Such antibodies may be also generated using variants, homologues or fragments of peptide sequences of SEQ ID NO:2, or any other immunogenic peptide sequences or immunogenic fragments thereof, which meet the following criteria:

(i) being a contiguous amino acid sequence of at least 6 amino acids, and
(ii) comprising at least 3 contiguous amino acid residues of any of the sequences SEQ ID NO:2.

The antibodies may also be produced in vivo by the individual to be treated, for example, by administering an immunogenic fragment according to the invention to said individual. Accordingly, the present invention further relates to a vaccine comprising an immunogenic fragment described above.

Inhibitory small molecules

In yet another embodiment the selective inhibitor according to the invention is a small molecule. Said small molecule may be identified by screening small molecule libraries for compounds capable of being antagonists for PACAP to PAC1 receptor, for example in an assay as described herein. Examples of small molecules are hydrazides, such as the compounds shown below:
In one embodiment the compound is
Pharmaceutical composition

An aspect of the invention is a pharmaceutical composition comprising an effective amount of at least one selective PAC1 inhibitor as defined herein or a salt thereof, optionally further comprising a pharmaceutically acceptable carrier substance.

Another aspect of the invention is a method for treating or alleviating a disorder or disease of a living animal body, including a human, where the disorder or disease is responsive to inhibition of PAC1, and where the method comprises administering to such a living animal body a therapeutically-effective amount of a selective PAC1 inhibitor as defined elsewhere herein.

Administration forms

The main routes of drug delivery, in the treatment method are intravenous, oral, and topical, as will be described below. Other drug-administration methods, such as subcutaneous injection or via inhalation, which are effective to deliver the drug to a target site or to introduce the drug into the bloodstream, are also contemplated.

The mucosal membrane to which the pharmaceutical preparation of the invention is administered may be any mucosal membrane of the mammal to which the biologically active substance is to be given, e.g. in the nose, vagina, eye, mouth, genital tract, lungs, gastrointestinal tract, or rectum, preferably the mucosa of the nose, mouth or vagina.

Compounds of the invention may be administered parenterally, that is by intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. Appropriate dosage forms for such
administration may be prepared by conventional techniques. The compounds may also be administered by inhalation, that is by intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques.

The compounds according to the invention may be administered with at least one other compound. The compounds may be administered simultaneously, either as separate formulations or combined in a unit dosage form, or administered sequentially.

**Dosing regimes**

The dosage requirements will vary with the particular drug composition employed, the route of administration and the particular subject being treated. Ideally, a patient to be treated by the present method will receive a pharmaceutically effective amount of the compound in the maximum tolerated dose, generally no higher than that required before drug resistance develops.

For all methods of use disclosed herein for the compounds, the daily oral dosage regimen will preferably be from about 0.01 to about 80 mg/kg of total body weight. The daily parenteral dosage regimen about 0.001 to about 80 mg/kg of total body weight. The daily topical dosage regimen will preferably be from 0.1 mg to 150 mg, administered one to four, preferably two or three times daily. The daily inhalation dosage regimen will preferably be from about 0.01 mg/kg to about 1 mg/kg per day. It will also be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound or a pharmaceutically acceptable salt thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optiumms can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound or a pharmaceutically acceptable salt thereof given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.
The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of a compound, alone or in combination with other agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle. The specifications for the unit dosage forms of the present invention depend on the particular compound or compounds employed and the effect to be achieved, as well as the pharmacodynamics associated with each compound in the host. The dose administered should be an "effective amount" or an amount necessary to achieve an "effective level" in the individual patient.

Since the "effective level" is used as the preferred endpoint for dosing, the actual dose and schedule can vary, depending on interindividual differences in pharmacokinetics, drug distribution, and metabolism. The "effective level" can be defined, for example, as the blood or tissue level desired in the patient that corresponds to a concentration of one or more compounds according to the invention.

In an embodiment the selective PAC1 inhibitor is used for the manufacture of a medicament, where the selective PAC1 inhibitor is in a concentration corresponding to a concentration of between 1nM ml⁻¹ serum and 500 µM ml⁻¹ serum of the individual to be treated with the medicament. The concentration may be between 1nM ml⁻¹ and 10 nM ml⁻¹, such as between 10 nM ml⁻¹ and 100 nM ml⁻¹, such as between 100 nM ml⁻¹ and 200 nM ml⁻¹, such as between 200 nM ml⁻¹ and 300 nM ml⁻¹, such as between 300 nM ml⁻¹ and 400 nM ml⁻¹, such as between 400 nM ml⁻¹ and 500 nM ml⁻¹, such as between 500 nM ml⁻¹ and 600 nM ml⁻¹, such as between 600 nM ml⁻¹ and 700 nM ml⁻¹, such as between 700 nM ml⁻¹ and 800 nM ml⁻¹, such as between 800 nM ml⁻¹ and 1000 nM ml⁻¹, such as between 1 µM ml⁻¹ and 100 µM ml⁻¹, such as between 100 µM ml⁻¹ and 200 µM ml⁻¹, such as between 200 µM ml⁻¹ and 300 µM ml⁻¹, such as between 300 µM ml⁻¹ and 400 µM ml⁻¹, such as between 400 µM ml⁻¹ and 500 µM ml⁻¹.

Pharmaceutical compositions containing a compound of the present invention may be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy 1995, edited by E. W. Martin, Mack Publishing
Company, 19th edition, Easton, Pa. The compositions may appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions or topical applications.

Formulations

Whilst it is possible for the compounds or salts of the present invention to be administered as the raw chemical, it is preferred to present them in the form of a pharmaceutical formulation. Accordingly, the present invention further provides a pharmaceutical formulation, for medicinal application, which comprises a compound of the present invention or a pharmaceutically acceptable salt thereof, as herein defined, and a pharmaceutically acceptable carrier therefore.

The compounds of the present invention may be formulated in a wide variety of oral administration dosage forms. The pharmaceutical compositions and dosage forms may comprise the compounds of the invention or its pharmaceutically acceptable salt or a crystal form thereof as the active component. The pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, wetting agents, tablet disintegrating agents, or an encapsulating material.

Preferably, the composition will be about 0.5% to 75% by weight of a compound or compounds of the invention, with the remainder consisting of suitable pharmaceutical excipients. For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like.

In powders, the carrier is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain from one to about seventy percent of the active compound. Suitable carriers are magnesium
carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term “preparation” is intended to include the formulation of the active compound with encapsulating material as carrier providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be as solid forms suitable for oral administration.

Drops according to the present invention may comprise sterile or non-sterile aqueous or oil solutions or suspensions, and may be prepared by dissolving the active ingredient in a suitable aqueous solution, optionally including a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100 degree C. for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container aseptically. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavours, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

Other forms suitable for oral administration include liquid form preparations including emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, toothpaste, gel dentrifice, chewing gum, or solid form preparations which are intended to be converted shortly before use to liquid form preparations. Emulsions may be prepared in solutions in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan monooleate, or acacia. Aqueous
solutions can be prepared by dissolving the active component in water and adding suitable colorants, flavours, stabilizing and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known suspending agents. Solid form preparations include solutions, suspensions, and emulsions, and may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

The compounds of the present invention may be formulated for parenteral administration (e.g., by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Examples of oily or nonaqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters (e.g., ethyl oleate), and may contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water.

Oils useful in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils useful in such formulations include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides; (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c)
nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-beta.-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

The parenteral formulations typically will contain from about 0.5 to about 25% by weight of the active ingredient in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5 to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

The compounds of the invention can also be delivered topically. Regions for topical administration include the skin surface and also mucous membrane tissues of the vagina, rectum, nose, mouth, and throat. Compositions for topical administration via the skin and mucous membranes should not give rise to signs of irritation, such as swelling or redness.

The topical composition may include a pharmaceutically acceptable carrier adapted for topical administration. Thus, the composition may take the form of a suspension, solution, ointment, lotion, sexual lubricant, cream, foam, aerosol, spray, suppository, implant, inhalant, tablet, capsule, dry powder, syrup, balm or lozenge, for example. Methods for preparing such compositions are well known in the pharmaceutical industry.

The compounds of the present invention may be formulated for topical administration to the epidermis as ointments, creams or lotions, or as a transdermal
patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also containing one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or colouring agents. Formulations suitable for topical administration in the mouth include lozenges comprising active agents in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a polyoxyethylene derivative thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Transdermal Delivery

The pharmaceutical agent-chemical modifier complexes described herein can be administered transdermally. Transdermal administration typically involves the delivery of a pharmaceutical agent for percutaneous passage of the drug into the systemic circulation of the patient. The skin sites include anatomic regions for transdermally administering the drug and include the forearm, abdomen, chest, back, buttock, mastoidal area, and the like.
Transdermal delivery is accomplished by exposing a source of the complex to a patient's skin for an extended period of time. Transdermal patches have the added advantage of providing controlled delivery of a pharmaceutical agent-chemical modifier complex to the body. See Transdermal Drug Delivery: Developmental Issues and Research Initiatives, Hadgraft and Guy (eds.), Marcel Dekker, Inc., (1989); Controlled Drug Delivery: Fundamentals and Applications, Robinson and Lee (eds.), Marcel Dekker Inc., (1987); and Transdermal Delivery of Drugs, Vols. 1-3, Kydonieus and Berner (eds.), CRC Press, (1987). Such dosage forms can be made by dissolving, dispersing, or otherwise incorporating the pharmaceutical agent-chemical modifier complex in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the compound in a polymer matrix or gel.

Passive Transdermal Drug Delivery

A variety of types of transdermal patches will find use in the methods described herein. For example, a simple adhesive patch can be prepared from a backing material and an acrylate adhesive. The pharmaceutical agent-chemical modifier complex and any enhancer are formulated into the adhesive casting solution and allowed to mix thoroughly. The solution is cast directly onto the backing material and the casting solvent is evaporated in an oven, leaving an adhesive film. The release liner can be attached to complete the system.

Alternatively, a polyurethane matrix patch can be employed to deliver the pharmaceutical agent-chemical modifier complex. The layers of this patch comprise a backing, a polyurethane drug/enhancer matrix, a membrane, an adhesive, and a release liner. The polyurethane matrix is prepared using a room temperature curing polyurethane prepolymer. Addition of water, alcohol, and complex to the prepolymer results in the formation of a tacky firm elastomer that can be directly cast only the backing material.

A further embodiment of this invention will utilize a hydrogel matrix patch. Typically, the hydrogel matrix will comprise alcohol, water, drug, and several hydrophilic
polymers. This hydrogel matrix can be incorporated into a transdermal patch between the backing and the adhesive layer.

The liquid reservoir patch will also find use in the methods described herein. This patch comprises an impermeable or semipermeable, heat sealable backing material, a heat sealable membrane, an acrylate based pressure sensitive skin adhesive, and a siliconized release liner. The backing is heat sealed to the membrane to form a reservoir which can then be filled with a solution of the complex, enhancers, gelling agent, and other excipients.

Foam matrix patches are similar in design and components to the liquid reservoir system, except that the gelled pharmaceutical agent-chemical modifier solution is constrained in a thin foam layer, typically a polyurethane. This foam layer is situated between the backing and the membrane which have been heat sealed at the periphery of the patch.

For passive delivery systems, the rate of release is typically controlled by a membrane placed between the reservoir and the skin, by diffusion from a monolithic device, or by the skin itself serving as a rate-controlling barrier in the delivery system. See U.S. Pat. Nos. 4,816,258; 4,927,408; 4,904,475; 4,588,580; 4,788,062; and the like. The rate of drug delivery will be dependent, in part, upon the nature of the membrane. For example, the rate of drug delivery across membranes within the body is generally higher than across dermal barriers. The rate at which the complex is delivered from the device to the membrane is most advantageously controlled by the use of rate-limiting membranes which are placed between the reservoir and the skin. Assuming that the skin is sufficiently permeable to the complex (i.e., absorption through the skin is greater than the rate of passage through the membrane), the membrane will serve to control the dosage rate experienced by the patient.

Suitable permeable membrane materials may be selected based on the desired degree of permeability, the nature of the complex, and the mechanical considerations related to constructing the device. Exemplary permeable membrane materials include a wide variety of natural and synthetic polymers, such as polydimethylsiloxanes (silicone rubbers), ethylenevinylacetate copolymer (EVA), polyurethanes, polyurethane-polyether copolymers, polyethylenes, polyamides,
polyvinylchlorides (PVC), polypropylenes, polycarbonates, polytetrafluoroethylenes (PTFE), cellulosic materials, e.g., cellulose triacetate and cellulose nitrate/acetate, and hydrogels, e.g., 2-hydroxyethylmethacrylate (HEMA).

Other items may be contained in the device, such as other conventional components of therapeutic products, depending upon the desired device characteristics. For example, the compositions according to this invention may also include one or more preservatives or bacteriostatic agents, e.g., methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chlorides, and the like. These pharmaceutical compositions also can contain other active ingredients such as antimicrobial agents, particularly antibiotics, anesthetics, analgesics, and antipruritic agents.

The compounds of the present invention may be formulated for administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the active component is dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and to solidify.

The active compound may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%].

The compounds of the present invention may be formulated for nasal administration. The solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided in a single or multidose form. In the latter case of a dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomizing spray pump.

The compounds of the present invention may be formulated for aerosol administration, particularly to the respiratory tract and including intranasal administration. The compound will generally have a small particle size for example of the order of 5 microns or less. Such a particle size may be obtained by means
known in the art, for example by micronization. The active ingredient is provided in a pressurized pack with a suitable propellant such as a chlorofluorocarbon (CFC) for example dichlorodifluoromethane, trichlorofluoromethane, or dichlorotetrafluoroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by a metered valve. Alternatively the active ingredients may be provided in a form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidone (PVP). The powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of e.g., gelatin or blister packs from which the powder may be administered by means of an inhaler.

When desired, formulations can be prepared with enteric coatings adapted for sustained or controlled release administration of the active ingredient.

The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

**Pharmacetically acceptable salts**

Pharmacetically acceptable salts of the instant compounds, where they can be prepared, are also intended to be covered by this invention. These salts will be ones which are acceptable in their application to a pharmaceutical use. By that it is meant that the salt will retain the biological activity of the parent compound and the salt will not have untoward or deleterious effects in its application and use in treating diseases.

Pharmacetically acceptable salts are prepared in a standard manner. If the parent compound is a base it is treated with an excess of an organic or inorganic acid in a
suitable solvent. If the parent compound is an acid, it is treated with an inorganic or organic base in a suitable solvent.

The compounds of the invention may be administered in the form of an alkali metal or earth alkali metal salt thereof, concurrently, simultaneously, or together with a pharmaceutically acceptable carrier or diluent, especially and preferably in the form of a pharmaceutical composition thereof, whether by oral, rectal, or parenteral (including subcutaneous) route, in an effective amount.

Examples of pharmaceutically acceptable acid addition salts for use in the present inventive pharmaceutical composition include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, p-toluenesulphonic acids, and arylsulphonic, for example.

Examples of a typical tablet

A typical tablet which may be prepared by conventional tabletting techniques may contain:

Core:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active compound (as free compound or salt thereof)</td>
<td>100 mg</td>
</tr>
<tr>
<td>Colloidal silicon dioxide (Aerosil)</td>
<td>1.5 mg</td>
</tr>
<tr>
<td>Cellulose, microcryst. (Avicel)</td>
<td>70 mg</td>
</tr>
<tr>
<td>Modified cellulose gum (Ac-Di-Sol)</td>
<td>7.5 mg</td>
</tr>
</tbody>
</table>

Magnesium stearate

Coating:
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMC</td>
<td>9 mg</td>
</tr>
<tr>
<td>'Mywacett 9-40 T</td>
<td>0.9 mg</td>
</tr>
</tbody>
</table>

*Acylated monoglyceride used as plasticizer for film coating.

10 The pharmaceutical carrier

Illustrative solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. A solid carrier can include one or more substances which may also act as flavouring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions, and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidone, low melting waxes and ion exchange resins.

Illustrative liquid carriers include syrup, peanut oil, olive oil, water, etc. Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium
carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carders are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant. Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

The carrier or excipient may include time delay material well known to the art, such as glyceryl monostearate or glyceryl distearate along or with a wax, ethylcellulose, hydroxypropylmethylcellulose, methylmethacrylate and the like. When formulated for oral administration, 0.01% Tween 80 in PHOSAL PG-50 (phospholipid concentrate with 1,2-propylene glycol, A. Nattermann & Cie. GmbH) has been recognized as providing an acceptable oral formulation for other compounds, and may be adapted to formulations for various compounds of this invention.

**Controlled release formulations**

The following terms may be considered to be substantially equivalent to controlled release, for the purposes of the present invention: continuous release, controlled release, delayed release, depot, gradual release, long-term release, programmed release, prolonged release, proportionate release, protracted release, repository, retard, slow release, spaced release, sustained release, time coat, timed release, delayed action, extended action, layered-time action, long acting, prolonged action, repeated action, slowing acting, sustained action, sustained-action medications, and extended release. Further discussions of these terms may be found in Lesczek Krowczynski, Extended-Release Dosage Forms, 1987 (CRC Press, Inc.).

The various controlled release technologies cover a very broad spectrum of drug dosage forms. Controlled release technologies include, but are not limited to physical systems and chemical systems.
Physical systems include, but not limited to, reservoir systems with rate-controlling membranes, such as microencapsulation, macroencapsulation, and membrane systems; reservoir systems without rate-controlling membranes, such as hollow fibers, ultra microporous cellulose triacetate, and porous polymeric substrates and foams; monolithic systems, including those systems physically dissolved in non-porous, polymeric, or elastomeric matrices (e.g., non-erodible, erodible, environmental agent ingestion, and degradable), and materials physically dispersed in non-porous, polymeric, or elastomeric matrices (e.g., non-erodible, erodible, environmental agent ingestion, and degradable); laminated structures, including reservoir layers chemically similar or dissimilar to outer control layers; and other physical methods, such as osmotic pumps, or adsorption onto ion-exchange resins.

Chemical systems include, but are not limited to, chemical erosion of polymer matrices (e.g., heterogeneous, or homogeneous erosion), or biological erosion of a polymer matrix (e.g., heterogeneous, or homogeneous). Additional discussion of categories of systems for controlled release may be found in Agis F. Kydonieus, Controlled Release Technologies: Methods, Theory and Applications, 1980 (CRC Press, Inc.).

Controlled release drug delivery systems may also be categorized under their basic technology areas, including, but not limited to, rate-preprogrammed drug delivery systems, activation-modulated drug delivery systems, feedback-regulated drug delivery systems, and site-targeting drug delivery systems.

In rate-preprogrammed drug delivery systems, release of drug molecules from the delivery systems "preprogrammed" at specific rate profiles. This may be accomplished by system design, which controls the molecular diffusion of drug molecules in and/or across the barrier medium within or surrounding the delivery system. Fick's laws of diffusion are often followed.

In activation-modulated drug delivery systems, release of drug molecules from the delivery systems is activated by some physical, chemical or biochemical processes.
and/or facilitated by the energy supplied externally. The rate of drug release is then controlled by regulating the process applied, or energy input.

In feedback-regulated drug delivery systems, release of drug molecules from the delivery systems may be activated by a triggering event, such as a biochemical substance, in the body. The rate of drug release is then controlled by the concentration of triggering agent detected by a sensor in the feedback regulated mechanism.

In a site-targeting controlled-release drug delivery system, the drug delivery system targets the active molecule to a specific site or target tissue or cell. This may be accomplished, for example, by a conjugate including a site specific targeting moiety that leads the drug delivery system to the vicinity of a target tissue (or cell), a solubilizer that enables the drug delivery system to be transported to and preferentially taken up by a target tissue, and a drug moiety that is covalently bonded to the polymer backbone through a spacer and contains a cleavable group that can be cleaved only by a specific enzyme at the target tissue.

While a preferable mode of controlled release drug delivery will be oral, other modes of delivery of controlled release compositions according to this invention may be used. These include mucosal delivery, nasal delivery, ocular delivery, transdermal delivery, parenteral controlled release delivery, vaginal delivery, rectal delivery and intrauterine delivery. All of these dosage forms may be manufactured using conventional techniques, together with the techniques discussed herein.

There are a number of controlled release drug formulations that are developed preferably for oral administration. These include, but are not limited to, osmotic pressure-controlled gastrointestinal delivery systems; hydrodynamic pressure-controlled gastrointestinal delivery systems; membrane permeation-controlled gastrointestinal delivery systems, which include microporous membrane permeation-controlled gastrointestinal delivery devices; gastric fluid-resistant intestine targeted controlled-release gastrointestinal delivery devices; gel diffusion-controlled gastrointestinal delivery systems; and ion-exchange-controlled gastrointestinal delivery systems, which include cationic and anionic drugs.

Additional information regarding controlled release drug delivery systems may be
found in Yie W. Chien, Novel Drug Delivery Systems, 1992 (Marcel Dekker, Inc.). Some of the formulations will now be discussed in more detail.

Enteric coatings may be applied to tablets to prevent the release of drugs in the stomach either to reduce the risk of unpleasant side effects or to maintain the stability of the drug which might otherwise be subject to degradation of expose to the gastric environment. Most polymers that are used for this purpose are polyacids that function by virtue of the fact that their solubility in aqueous medium is pH-dependent, and they require conditions with a pH higher then normally encountered in the stomach.

Enteric coatings may be used to coat a solid or liquid dosage form of the compounds according to the invention. Enteric coatings promote the inventive compounds remaining physically incorporated in the dosage form for a specified period when exposed to gastric juice. Yet the enteric coatings are designed to disintegrate in intestinal fluid for ready absorption. Delay of the compounds' absorption is dependent on the rate of transfer through the gastrointestinal tract, and so the rate of gastric emptying is an important factor. Some investigators have reported that a multiple-unit type dosage form, such as granules, may be superior to a single-unit type. Therefore, in a preferable embodiment, the compounds according to the invention may be contained in an enterically coated multiple-unit dosage form. In a more preferable embodiment, the dosage form of the compounds according to the invention is prepared by spray-coating granules of an compounds-enteric coating agent solid dispersion on an inert core material. These granules can result in prolonged absorption of the drug with good bioavailability.

Typical enteric coating agents include, but are not limited to, hyd roxypropylmethylcellulose phthalate, methacrylic acid-methacrylic acid ester copolymer, polyvinyl acetate-phthalate and cellulose acetate phthalate. Akihiko Hasegawa, Application of solid dispersions of Nifedipine with enteric coating agent to prepare a sustained-release dosage form, Chem. Pharm. Bull. 33: 1615-1619 (1985). Various enteric coating materials may be selected on the basis of testing to achieve an enteric coated dosage form designed ab initio to have a preferable combination of dissolution time, coating thicknesses and diametral crushing strength. S. C. Porter et al., The Properties of Enteric Tablet Coatings Made From

On occasion, the performance of an enteric coating may hinge on its permeability.


Another type of useful oral controlled release structure is a solid dispersion. A solid dispersion may be defined as a dispersion of one or more active ingredients in an inert carrier or matrix in the solid state prepared by the melting (fusion), solvent, or melting-solvent method. Akihiko Hasegawa, Super Saturation Mechanism of Drugs from Solid Dispersions with Enteric Coating Agents, Chem. Pharm. Bull. 36: 4941-4950 (1998). The solid dispersions may be also called solid-state dispersions. The term "coprecipitates" may also be used to refer to those preparations obtained by the solvent methods.

Solid dispersions may be used to improve the solubilities and/or dissolution rates of compounds according to the invention that may be poorly water-soluble. See generally Hiroshi Yuasa, et al., Application of the Solid Dispersion Method to the Controlled Release Medicine. III. Control of the Release Rate of Slightly Water-Soluble Medicine From Solid Dispersion Granules, Chem. Pharm. Bull. 41:397-399 (1993). The solid dispersion method was originally used to enhance the dissolution rate of slightly water-soluble medicines by dispersing the medicines into water-soluble carriers such as polyethylene glycol or polyvinylpyrrolidone, Hiroshi Yuasa, et al., Application of the Solid Dispersion Method to the Controlled Release of Medicine. IV. Precise Control of the Release Rate of a Water-Soluble Medicine by Using the Solid Dispersion Method Applying the Difference in the Molecular Weight of a Polymer, Chem. Pharm. Bull. 41:933-936 (1993).
The selection of the carrier may have an influence on the dissolution characteristics of the dispersed drug because the dissolution rate of a component from a surface may be affected by other components in a multiple component mixture. For example, a water-soluble carrier may result in a fast release of the drug from the matrix, or a poorly soluble or insoluble carrier may lead to a slower release of the drug from the matrix. The solubility of poorly water soluble compounds according to the invention may also be increased owing to some interaction with the carriers.

Examples of carriers useful in solid dispersions according to the invention include, but are not limited to, water-soluble polymers such as polyethylene glycol, polyvinylpyrrolidone, or hydroxypropylmethyl-cellulose. Akihiko Hasegawa, Application of Solid Dispersions of Nifedipine with Enteric Coating Agent to Prepare a Sustained-release Dosaae Form, Chem. Pharm. Bull. 33:1615-1619 (1985).

There are various methods commonly known for preparing solid dispersions. These include, but are not limited to the melting method, the solvent method and the melting-solvent method.

In the melting method, the physical mixture of a drug in a water-soluble carrier is heated directly until it melts. The melted mixture is then cooled and solidified rapidly while rigorously stirred. The final solid mass is crushed, pulverized and sieved. Using this method a super saturation of a solute or drug in a system can often be obtained by quenching the melt rapidly from a high temperature. Under such conditions, the solute molecule may be arrested in solvent matrix by the instantaneous solidification process. A disadvantage is that many substances, either drugs or carriers, may decompose or evaporate during the fusion process at high temperatures. However, this evaporation problem may be avoided if the physical mixture is heated in a sealed container. Melting under a vacuum or blanket of an inert gas such as nitrogen may be employed to prevent oxidation of the drug or carrier.

The solvent method has been used in the preparation of solid solutions or mixed crystals of organic or inorganic compounds. Solvent method dispersions may be prepared by dissolving a physical mixture of two solid components in a common solvent, followed by evaporation of the solvent. The main advantage of the solvent
method is that thermal decomposition of drugs or carriers may be prevented because of the low temperature required for the evaporation of organic solvents. However, some disadvantages associated with this method are the higher cost of preparation, the difficulty in completely removing liquid solvent, the possible adverse effect of its supposedly negligible amount of the solvent on the chemical stability of the drug.

Another method of producing solid dispersions is the melting-solvent method. It is possible to prepare solid dispersions by first dissolving a drug in a suitable liquid solvent and then incorporating the solution directly into a melt of polyethylene glycol, obtainable below 70 degrees, without removing the liquid solvent. The selected solvent or dissolved adenosine analogs may be selected such that the solution is not miscible with the melt of polyethylene glycol. The polymorphic form of the adenosine analogs may then be precipitated in the melt. Such a unique method possesses the advantages of both the melting and solvent methods. Win Loung Chiou, et al., Pharmaceutical Applications of Solid Dispersion Systems, J. Pharm. Sci. 60:1281-1301 (1971).

Another controlled release dosage form is a complex between an ion exchange resin and the compounds according to the invention. Ion exchange resin-drug complexes have been used to formulate sustained-release products of acidic and basic drugs. In one preferable embodiment, a polymeric film coating is provided to the ion exchange resin-drug complex particles, making drug release from these particles diffusion controlled. See Y. Raghunathan et al., Sustained-released drug delivery system I: Coded ion-exchange resin systems for phenylpropanolamine and other drugs, J. Pharm. Sciences 70: 379-384 (1981).

Injectable micro spheres are another controlled release dosage form. Injectable micro spheres may be prepared by non-aqueous phase separation techniques, and spray-drying techniques. Micro spheres may be prepared using polylactic acid or copoly(lactic/glycolic acid). Shigeyuki Takada, Utilization of an Amorphous Form of a Water-Soluble GPIIb/IIIa Antagonist for Controlled Release From Biodegradable Micro spheres, Pharm. Res. 14:1 146-1 150 (1997), and ethyl cellulose, Yoshiyuki Koida, Studies on Dissolution Mechanism of Drugs from Ethyl Cellulose Microcapsules, Chem. Pharm. Bull. 35:1538-1545 (1987).
Other controlled release technologies that may be used in the practice of this invention are quite varied. They include SODAS (Spheroidal Oral Drug Absorption System), INDAS (Insoluble Drug Absorption System), IPDAS (Intestinal Protective Drug Absorption System), MODAS (Multiporous Oral Drug Absorption System), EFVAS (Effervescent Drug Absorption System), PRODAS (Programmable Oral Drug Absorption System), and DUREDAS (Dual Release Drug Absorption System) available from Elan Pharmaceutical Technologies, Dublin, Ireland. SODAS are multi particulate dosage forms utilizing controlled release beads. INDAS are a family of drug delivery technologies designed to increase the solubility of poorly soluble drugs. IPDAS are multi particulate tablet formation utilizing a combination of high density controlled release beads and an immediate release granulate. MODAS are controlled release single unit dosage forms. Each tablet consists of an inner core surrounded by a semipermeable muciparous membrane that controls the rate of drug release. EFVAS is an effervescent drug absorption system, PRODAS is a family of multi particulate formulations utilizing combinations of immediate release and controlled release mini-tablets. DUREDAS is a bilayer tablet formulation providing dual release rates within the one dosage form. Although these dosage forms are known to one of skill, certain of these dosage forms will now be discussed in more detail.

INDAS was developed specifically to improve the solubility and absorption characteristics of poorly water soluble drugs. Solubility and, in particular, dissolution within the fluids of the gastrointestinal tract is a key factor in determining the overall oral bioavailability of poorly water soluble drug. By enhancing solubility, one can increase the overall bioavailability of a drug with resulting reductions in dosage. INDAS takes the form of a high energy matrix tablet. In a preferred embodiment of the invention production involves including adenosine analogs in an amorphous form together with a combination of energy, excipients, and unique processing procedures.

Once included in the desirable physical form, the resultant high energy complex may be stabilized by an absorption process that utilizes a novel polymer cross-linked technology to prevent recrystallization. The combination of the change in the physical state of the adenosine analogs according to the invention coupled with the
solubilizing characteristics of the excipients employed enhances the solubility of the adenosine analogs according to the invention. The resulting absorbed amorphous drug complex granulate may be formulated with a gel-forming erodable tablet system to promote substantially smooth and continuous absorption.

5

IPDAS is a multiparticulate tablet technology that may enhance the gastrointestinal tolerability of potential irritant and ulcerogenic drugs. Intestinal protection is facilitated by the multiparticulate nature of the IPDAS formulation which promotes dispersion of an irritant adenosine analog according to the invention throughout the gastrointestinal tract. Controlled release characteristics of the individual beads may avoid high concentration of drug being both released locally and absorbed systemically. The combination of both approaches serves to minimize the potential harm of the adenosine analog according to the invention with resultant benefits to patients.

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IPDAS is composed of numerous high density controlled release beads. Each bead may be manufactured by a two step process that involves the initial production of a micromatrix with embedded adenosine analogs according to the invention and the subsequent coating of this micromatrix with polymer solutions that form a rate limiting semipermeable membrane in vivo. Once an IPDAS tablet is ingested, it may disintegrate and liberate the beads in the stomach. These beads may subsequently pass into the duodenum and along the gastrointestinal tract, preferably in a controlled and gradual manner, independent of the feeding state. Adenosine analog release occurs by diffusion process through the micromatrix and subsequently through the pores in the rate controlling semipermeable membrane. The release rate from the IPDAS tablet may be customized to deliver a drug-specific absorption profile associated with optimized clinical benefit. Should a fast onset of activity be necessary, immediate release granulate may be included in the tablet. The tablet may be broken prior to administration, without substantially compromising drug release, if a reduced dose is required for individual titration.

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MODAS is a drug delivery system that may be used to control the absorption of water soluble adenosine analogs according to the invention. Physically MODAS is a non-disintegrating tablet formulation that manipulates drug release by a process of rate limiting diffusion by a semipermeable membrane formed in vivo. The diffusion
process essentially dictates the rate of presentation of drug to the gastrointestinal fluids, such that the uptake into the body is controlled. Because of the minimal use of excipients, MODAS can readily accommodate small dosage size forms. Each MODAS tablet begins as a core containing active drug plus excipients. This core is coated with a solution of insoluble polymers and soluble excipients. Once the tablet is ingested, the fluid of the gastrointestinal tract may dissolve the soluble excipients in the outer coating leaving substantially the insoluble polymer. What results is a network of tiny, narrow channels connecting fluid from the gastrointestinal tract to the inner drug core of water soluble drug. This fluid passes through these channels, into the core, dissolving the drug, and the resultant solution of drug may diffuse out in a controlled manner. This may permit both controlled dissolution and absorption. An advantage of this system is that the drug releasing pores of the tablet are distributed over substantially the entire surface of the tablet. This facilitates uniform drug absorption and reduces aggressive unidirectional drug delivery. MODAS represents a very flexible dosage form in that both the inner core and the outer semipermeable membrane may be altered to suit the individual delivery requirements of a drug. In particular, the addition of excipients to the inner core may help to produce a micro environment within the tablet that facilitates more predictable release and absorption rates. The addition of an immediate release outer coating may allow for development of combination products.

Additionally, PRODAS may be used to deliver adenosine analogs according to the invention. PRODAS is a multi particulate drug delivery technology based on the production of controlled release mini tablets in the size range of 1.5 to 4 mm in diameter. The PRODAS technology is a hybrid of multi particulate and hydrophilic matrix tablet approaches, and may incorporate, in one dosage form, the benefits of both these drug delivery systems.

In its most basic form, PRODAS involves the direct compression of an immediate release granulate to produce individual mini tablets that contain adenosine analogs according to the invention. These mini tablets are subsequently incorporated into hard gels and capsules that represent the final dosage form. A more beneficial use of this technology is in the production of controlled release formulations. In this case, the incorporation of various polymer combinations within the granulate may delay the release rate of drugs from each of the individual mini tablets. These mini tablets
may subsequently be coated with controlled release polymer solutions to provide additional delayed release properties. The additional coating may be necessary in the case of highly water soluble drugs or drugs that are perhaps gastroirritants where release can be delayed until the formulation reaches more distal regions of the gastrointestinal tract.

The pharmaceutical composition of the invention comprising a channel inhibitor may contain between 0.0001 to 90 % by volume of the compound having channel inhibitor activity.

**Screening**

A further aspect of the invention is a method for screening for a compound for the treatment or alleviation of migraine and/or other headaches, said method comprising:

a. obtaining a compound being a possible selective PAC1 inhibitor,

b. measuring binding affinity to PAC1 of said possible selective PAC1 inhibitor,

c. measuring inhibitory effect in relation to PAC1 of said possible selective PAC1 inhibitor,

d. wherein said possible PAC1 inhibitor is determined to be a compound for treatment or alleviation of migraine and/or other headaches if said possible PAC1 inhibitor binds to PAC1 with high affinity, and when said PAC1 inhibitor has inhibitory effect in relation to PAC1.

After being identified the selective PAC1 inhibitor may be tested in a migraine or headache model. A human headache model is described in Example 3.
Detailed description of figures

**Figure 1A**: Median headache response (0 point verbal VAS scale) after 20 minute infusion of 10 pmol/kg/min PACAP38 or placebo in healthy volunteers. Significant headache was seen using PACAP38 compared to placebo to an extent similar to that of CGRP or NO.

**Figure 1B**: 10 pmol/kg/min PACAP38 or placebo in healthy volunteers. Significant headache was seen compared to placebo to an extent similar to that of CGRP or NO.

**Figure 2**: Median headache response (0 point verbal VAS scale) after 20 minute infusion of 10 pmol/kg/min VIP or placebo in healthy volunteers. Slightly more headache was seen using VIP compared to placebo to an extent similar to that of CGRP or NO.

**Examples**

The following examples are for illustrative purposes only and should not be construed as limiting the scope of the invention, which is defined by the appended claims.

**Example 1**

Binding affinity assay

**Principle:**
The ligand is incubated in logarithmically increasing concentrations with preparation of cell membranes naturally expressing the PAC1. Incubation with a radioactive labelled ligand is performed followed by washing.

The radioactivity is measured. The less radioactivity the better binding of the ligand. Percentage of total amount is plotted against concentration of the ligand.

and IC\(_{50}\) (the concentration inhibiting receptor binding by 50 %) may be calculated. The lower IC\(_{50}\), the better affinity.
Methods:

Brain tissue from a rat or rabbit is homogenised in icecooled homogenisation buffer with Polytron apparatus and centrifuged at 1000g in 10 minutes/4 °. The pellet is resuspended in buffer. The process is repeated. Pellets containing so-called crude membranes are incubated in 2 hours with buffer having added logarithmically increasing concentration of ligand as well as 50 pM 125PACAP-27. The solution is filtered through glass microfibre filters, and the radioactivity is measured using a counter. All tests are conducted three times.

Example 2

Functional assay for inhibitory effect

Principle

The examined receptors mediates their signal via an increase in cyclic adenosine monophosphate (cAMP). Cell cultures not naturally expressing the examined receptors are modified to express the receptor.

To the cell cultures are added logarithmically increasing concentrations of the examined ligand, whereupon incubation with a known agonist is performed. cAMP is measured. The less cAMP generated after addition of the known agonist, the better inhibition of the receptor by the ligand. Percentage inhibition is plotted against log. concentration of ligand and EC50. The lower EC50 the more effective the antagonist.

By comparing the effect on relevant receptors (in this instance human forms of VPAC1, VPAC2 og PAC1 as well as glucagon and secretin receptors) it is possible to determine the selectivity of the ligand. Normally a factor of 10 or more in difference in ED50 is desired in order to announce the ligand as selective.

Methods:
COS cells are incubated with plasmids containing recombinant cDNA encoding the receptor. Dimethylsulfoxide is added for a short period to break the membrane barrier so that vector may penetrate into the cell. Incubation is conducted for 3 days.
Cell cultures are added by pipette into plastic plates with wells and allowed to grow until the cells form a confluent layer. Stimulation with the ligand in question (increasing concentrations in different wells) as well as 1 nM PACAP-38. The process is stopped after exactly 10 minutes by adding icecooled TRIS and EDTA. The cells are harvested, cooked for 5 minutes and centrifuged for 5 minutes at high speed. The supernatants are collected and cAMP measured using a commercially available radioimmunoassay (Amersham ®). The tests are conducted three times.

Results:
Testing the two selective PAC1 inhibitors, PACAP6-38 and M65 according to this example, the following results were obtained:

M65 Antagonist 8.7 pIC50
PACAP(6-38) Antagonist 8.1-7.4 pIC50

Example 3

Induction of headache using PACAP38, VIP or placebo

In a human headache model (CGRP studie MODEL: Lassen LH, Haderslev PA, Jacobsen VB, Iversen HK, Sperling B, Olesen J., CGRP may play a causative role in migraine. Cephalalgia. 2002 Feb;22(1):54-61.) the effect of PACAP on headache was studied. A general description of the headache model is found in: Kruuse C, Thomsen LL, Birk S, Olesen J., Migraine can be induced by sildenafil without changes in middle cerebral artery diameter. Brain. 2003 Jan;126(Pt 1):241-7.

The results are shown in figures 1A, 1B and 2.

Example 4

Cell based assays for determining effect and receptor specificity of compounds / antagonists

Cloning and cell lines construction
The open reading frames of hPAC1 (herein PAC1), VPAC1 and VPAC2 receptors were amplified from the 3 plasmids EM7(pCDNA3-hPAC1-null vector), pCMV SPORT6-hVPAC1 (Acc# BC064424) and pCDNA3-hVPAC2 (Acc# X95097) respectively, using Phusion High-Fidelity DNA Polymerase (New England Biolab), and subcloned into the specific pX-9CRE-EYFP assay cloning vector.

The three constructed plasmids phPAC1-9CRE-EYFP, pVPAC1-9CRE-EYFP and pVPAC2-9CRE-EYFP were first doubly confirmed by restriction enzyme digestion and PCR amplification for the correct cloning of hPAC1, VPAC1 and VPAC2 genes and then transfected into HEK-293 cell line (ATCC#: CRL-1573). Three cell lines (hPAC1, VPAC1 and VPAC2) with stable expression of each receptor were obtained after continuously growing for two weeks under puromycin selection. 2.5 nM of agonist PACAP-38 activation experiments showed that the cells were not in complete uniform, some cells expressing a weak EYFP fluorescent signal and others expressing a very strong signal, however, under higher than 20 nM of PACAP-38 activation, nearly all cells expressed fluorescent signal.

Cell culture for assaying
The cell culture assaying was carried out in 96-well microplates (Nunc#1 60377). Each cell line (hPAC1, VPAC1 and VPAC2) was grown in 20 ml of Ham's F-12 Nutrient Mixture (Invitrogen #31 765) supplemented with Non Essential Amino Acids (Invitrogen #11140-035), 1 mM of Sodium pyruvate (Sigma #S8636), 10% of fetal bovine serum (FBS, Sigma #F7524), 100 U/ml of penicillin and 100 µg/ml of streptomycin (Penicillin-Streptomycin Solution, Sigma #P4333) to achieve 70-80% confluence in a T-75 flask (Nunc # 156499) in cell culture incubator at 37 °C and 5% CO2. The cells were washed with 10 ml of PBS buffer (Sigma #D8537), detached with 2 ml of Trypsin solution (Sigma #T4049) for 3 min in the incubator and suspended in the medium. For each cell line 20 ml cell suspension culture with a cell density of 1 x 105 cells/ml was used for assaying on one 96-well microplate and 200 µl of cells were added into each well (20,000 cells per well). After addition of antagonists and agonist, the cells were grown in the incubator for 16-20 hours before photo taking. From each well one photo showing EYFP fluorescent signalling and one photo showing cell numbers were taken, the fluorescent signal strength and the cell numbers on each photo were determined using the software Cell Image Analyzer.
The agonist PACAP-38 assay

12 concentration points with double increment were used in the PACAP-38 (Sigma#A1439, Pituitary adenylate cyclase activating polypeptide-38) assay. The control (without PACAP-38) assay was done in separate experiments. 1 µl of ddH2O dissolved PACAP-38 stock solutions with concentrations in two fold dilution rows were added into each well respectively, achieving final concentrations of 0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 nM. The assay at each concentration point was repeated three times.

The antagonist PACAP-6-27 assay

12 concentration points with double increment were used in PACAP-6-27 (Bachem A/G #H-8435) assay. 1 µl of ddH2O dissolved PACAP-6-27 stock solutions with concentrations in two fold dilution rows were added into each well respectively, achieving final concentrations of 0, 5, 9, 10, 20, 39, 78, 156, 313, 625, 1250, 2500 and 5000 nM. 10 minutes after addition of PACAP-6-27, 20 nM of PACAP-38 (0.8 µl of 5 µM ddH2O dissolved PACAP-38) was added into each well. The assay at each concentration point was repeated three times.

The antagonists compound assay

12 or more concentration points with double increment are used in the compound assays. A solvent, for example 0.5 µl of DMSO, dissolves the compound stock solutions with concentrations in two fold dilution rows are added into each well respectively, achieving final concentrations of the compound of for example: 0, 8, 16, 31, 62, 125, 250, 500, 1000, 2000, 4000 and 8000 nM; or for example: 23, 47, 94, 188, 375, 750, 1500, 3000, 6000, 12000 and 24000 nM. 10 minutes after addition of the compounds, 20 nM PACAP-38 (0.8 µl of 5 µM ddH2O dissolved PACAP-38) is added into each well. The solvent (for example: DMSO) concentration in the cell culture is 0.25 %. The assays at each concentration point are repeated three times.
Claims

1. Use of a selective PAC1 inhibitor for the manufacture of a medicament for treatment or alleviation of migraine and/or other headaches.

2. The use according to claim 1, wherein said selective PAC1 inhibitor specifically inhibits PAC1.

3. The use according to any of the preceding claims, wherein said selective PAC1 inhibitor binds to PAC1 with an affinity being at least 10 times higher than the inhibitor's binding affinity to VPAC1 or VPAC2.

4. The use according to any of the preceding claims, wherein said treatment is prophylactic.

5. The use according to any of the preceding claims 1-6, wherein said treatment is acute treatment.

6. The use according to any of the preceding claims, wherein said treatment is systemic.

7. A pharmaceutical composition comprising an effective amount of at least one selective PAC1 inhibitor as defined in any of the claims 1 to 6 or a salt thereof, optionally further comprising a pharmaceutically acceptable carrier substance.

8. A method for treating or alleviating a disorder or disease of a living animal body, including a human, which disorder or disease is responsive to inhibition of PAC1, which method comprises administering to such a living animal body a therapeutically-effective amount of a selective PAC1 inhibitor as defined in any of the claims 1 to 6.

9. The method according to claim 8, wherein the disease is migraine and/or headache.
10. A method for screening for a compound for the treatment or alleviation of migraine and/or other headaches, said method comprising:

obtaining a compound being a possible selective PAC1 inhibitor,

measuring binding affinity to PAC1 of said possible selective PAC1 inhibitor,

measuring inhibitory effect in relation to PAC1 of said possible selective PAC1 inhibitor,

wherein said possible PAC1 inhibitor is determined to be a compound for treatment or alleviation of migraine and/or other headaches if said possible PAC1 inhibitor binds to PAC1 with high affinity, and when said PAC1 inhibitor has inhibitory effect in relation to PAC1.
Fig. 2

![Graph showing VRS data during infusion of VIP and placebo.](image-url)