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(54) Title: PDE5 INHIBITORS FOR THE TREATMENT OF CANCER

(57) Abstract: The present invention relates to the treatment of cancer, particularly colon cancer, comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal, preferably a human, in need thereof. Preferably the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoq uinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

Background

Protein kinases ("PKs") are enzymes that catalyze the phosphorylation of hydroxy groups on tyrosine, serine and threonine residues of proteins. The consequences of this seemingly simple activity are staggering; cell growth, differentiation and proliferation, i.e., virtually all aspects of cell life in one way or another depend on PK activity. Furthermore, abnormal PK activity has been related to a host of disorders, ranging from relatively non-life threatening diseases such as psoriasis to extremely virulent diseases, such as glioblastoma (brain cancer).

The PKs can be conveniently broken down into two classes, the protein tyrosine kinases (PTKs) and the serine-threonine kinases.

One of the prime aspects of PTK activity is their involvement with growth factor receptors. Growth factor receptors are cell-surface proteins. When bound by a growth factor ligand, growth factor receptors are converted to an active form which interacts with proteins on the inner surface of a cell membrane. This leads to phosphorylation on tyrosine residues of the receptor and other proteins and to the formation inside the cell of complexes with a variety of cytoplasmic signaling molecules that, in turn, effect numerous cellular responses such as cell division (proliferation), cell differentiation, cell growth, expression of metabolic effects to the extracellular microenvironment, etc. For a more complete discussion, see Schlessinger and Ullrich, *Neuron*, Vol. 9, pp. 303-391 (1992).

A member of the tyrosine kinase growth factor receptor family is the vascular endothelial growth factor (VEGF) receptor subgroup. VEGF is a dimeric glycoprotein similar to platelet derived growth factor receptor but has different biological functions and target cell

specificity *in vivo*. In particular, VEGF is presently thought to play an essential role is vasculogenesis and angiogenesis.

VEGF induces endothelial cell proliferation and vascular permeability. VEGF is essential for establishment of angiogenesis in most solid tumors. VEGF has been identified as an HIF-1 target gene. See Semenza et al., *Novartis Foundation Symposium*, Vol. 240, pp. 251-264 (2001). HIF-1 activates VEGF, which induces angiogenesis leading to an increase in the vascular density and hence a decrease in the diffusion distance for oxygen. See Semenza et al. (2001), *supra*.

A particularly life-threatening form of cancer induced by VEGF is cancer of the colon. Cancer of the colon is a common and deadly disease in the Western world. Genetic predisposition plays an important role.

Phosphodiesterases (PDEs) can be subdivided into class I and class II [see *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Charbonneau et al., Eds, pp. 267-296, J. Wiley & Sons, Inc., NY (1990)], which have no recognizable sequence similarity. Class I include all known mammalian PDEs and consist of at least 10 families that are products of separate genes. See Corbin and Francis, *JBC*, Vol. 274, No. 20, pp. 13729-13732 (1999). Most families contain more than one gene and most genes code for more than one messenger RNA (mRNA), by alternative splicing or alternative transcriptional start sites. PDE4, PDE7 and PDE8 are highly specific for hydrolysis of cAMP, whereas PDE5, PDE6 and PDE9 are highly cGMP specific. PDE1, PDE2, PDE3 and PDE10 have mixed specificity.

Each PDE has a conserved catalytic domain of about 270 amino acids with a high degree of conservation (25-30%) of amino acid sequence among PDE families, which is located carboxyl-terminal to its regulatory domain. Cyclic nucleotides are degraded by PDE-catalyzed hydrolytic cleavage of the 3'-phosphodiester bond, resulting in formation of the corresponding inactive 5'-monophosphate.

Three different isoforms of PDE5A have been reported, PDE5A1, PDE5A2 and PDE5A3. All PDE5 variants differ only at the *N*-terminal end. PDE5A1 appears to be the predominant form expressed in most PDE5 containing tissues. PDE5A2 contains a significantly shorter amino acid *N*-terminal fragment and also has been shown in several species. PDE5A3 has been reported in human tissues only, based on RT-PCR data. See

Rybalkin et al., *Circ Res*, Vol. 93, pp. 280-291 (2003). PDE5 is highly specific for cGMP hydrolysis and contains two homologous *N*-terminal regulatory domains, recently defined as GAF A and GAF B. See Martinez et al., *Proc Natl Acad Sci USA*, Vol. 99, pp. 13260-13265 (2002). PDE5 is directly activated upon cGMP binding to its GAF A domain. Without cGMP bound, PDE5 is in a non-activated state. Only activated PDE5 is phosphorylated (Ser-92) by the cGMP-dependent protein kinase (PKG).

Cyclic nucleotides transduce signals through direct protein binding. The best characterized of these protein targets are the cyclic-nucleotide-dependent PKs, PKA and PKG. The cyclic nucleotide signaling cascade engages in cross-talk with several other critical signaling pathways and also undergoes autoregulation.

cGMP is an important regulator of smooth muscle function. Nitric oxide and other endogenous vasodilators regulate smooth muscle tone through the cGMP/PKG signaling pathway. PDE5 effectively controls the development of smooth muscle relaxation.

PDE inhibitors have been evaluated as potential antidepressants, anti-inflammatory agents, antiproliferative agents, antihypertensive and cardiovascular agents and cytoprotective agents.

Many of these inhibitors, such as caffeine and 3-isobutyl-1-methylxantine, are non-selective and inhibit many of the PDE families. To date, clinical development of selective PDE inhibitors has focused primarily on PDE3, PDE4 and PDE5.

All PDE5 inhibitors described so fare have been developed as competitive inhibitors at the active site. Because all PDEs share a high degree of sequence similarity in their catalytic domain, the selectivity profiles of most inhibitors at least partially overlap. For example the PDE5 inhibitor zaprinast is also a relatively good inhibitor of PDE1. The PDE5 inhibitor sildenafil can also inhibit the photoreceptor PDE6 in the nmol/L range of concentration. Tadalafil, a newer PDE5 inhibitor, has been reported as much more specific for PDE5 than for PDE6, but is a relatively good inhibitor of PDE11 (a summary of PDE inhibitors is listed in Table I of Essayan, *J Allergy Clin Immunol*, Vol. 108, No. 5, pp. 671-680 (2001).

The non-steroidal anti-inflammatory drug exisulind was described to regulate apoptosis via PKG-mediated beta (β)-catenin phosphorylation and subsequent β-catenin

degradation in colon cancer cells by inhibiting cGMP PDE of either the PDE2 or PDE5 isozyme families to cause a sustained increase in cGMP and the activation of PKG. See Thompson et al., *Cancer Res*, Vol. 60, pp. 3338-3342 (2000). The effect appears to require nonselective rather than selective PDE5 inhibitors. Furthermore, in human bladder tumor cells, the expression of PDE4 and PDE5 were detected, which were sensitive to exisulind treatment. See Piazza et al., *Cancer Res*, Vol. 61, pp. 3961-3968 (2001).

A potent inhibitory effect of selective inhibitors for cAMP-specific PDE2 (EHNA) and PDE4 (RP7340l) on VEGF-induced HUVEC proliferation, cell cycle distribution and migration was described recently. See Favot et al., *Thromb Haemost*, Vol. 90, pp. 334-343 (2003). Selective cytokine inhibitory drugs have been reported to be potent PDE-4 inhibitors that inhibit TNFalpha (α) production and are highly antiangiogenic. In addition, inhibition of PDE-4 induces apoptosis in human CLL lymphocytes. See Zeldis et al., *Semin Oncol*, Vol. 30, pp. 275-281 (2003). Sildenafil was reported to increase synthesis of VEGF and enhance angiogenesis in ischemic rat brain, 24 hours after stroke. See Zhang et al., *Circ Res*, Vol. 92, pp. 308-313 (2003).

Accordingly, there is a need for PDE5 inhibitors which may be employed to inhibit angiogenesis, vascularization and VEGF expression for the treatment of cancer, particularly colon cancer.

Summary of the Invention

In accordance with the above-mentioned objectives and others, the present invention provides a method of inhibiting angiogenesis comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal in need thereof.

In another aspect, the present invention provides a method of inhibiting vascularization comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal in need thereof.

In another aspect, the present invention provides a method of inhibiting VEGF expression comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal in need thereof.

In still another embodiment of the present invention, there is provided a method of treating cancer comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal in need thereof. In accordance with this aspect of the invention, the PDE5 inhibitors may be particularly useful in the treatment of colon cancer and preferably the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

In another aspect, the present invention provides a method of inhibiting VEGF expression comprising administering a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents to a warm-blooded animal in need thereof.

In another embodiment of this aspect of the present invention, there is provided a method treating cancer comprising administering a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents to a warm-blooded animal in need thereof. In accordance with this aspect of the invention, the PDE5 inhibitors may be particularly useful in the treatment of colon cancer and preferably the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor for the manufacture of a medicament for the inhibition of angiogenesis.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor for the manufacture of a medicament for the inhibition of vascularization.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor for the manufacture of a medicament for the inhibition of VEGF expression.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor for the manufacture of a medicament for the treatment of cancer.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor for the manufacture of a medicament for the treatment of colon cancer wherein preferably the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents for the manufacture of a medicament for the inhibition of VEGF expression.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents for the manufacture of a medicament for the treatment of cancer.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents for the manufacture of a medicament for the treatment of colon cancer.

In another aspect, the present invention relates to the use of a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents for the manufacture of a medicament for the treatment of colon cancer wherein the PDE5 inhibitor is preferably 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

In another aspect, the present invention relates to a pharmaceutical composition for the inhibition of angiogenesis comprising a therapeutically effective amount of a PDE5 inhibitor.

In another aspect, the present invention relates to a pharmaceutical composition for the inhibition of vascularization comprising a therapeutically effective amount of aPDE5 inhibitor.

In another aspect, the present invention relates to a pharmaceutical composition for the inhibition of VEGF expression comprising a therapeutically effective amount of a PDE5 inhibitor.

In another aspect, the present invention relates to a pharmaceutical composition comprising a therapeutically effective amount of a PDE5 inhibitor for the treatment of cancer.

In another aspect, the present invention relates to a pharmaceutical composition comprising a therapeutically effective amount of a PDE5 inhibitor for the treatment of colon cancer wherein preferably the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

In another aspect, the present invention relates to a pharmaceutical composition comprising a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents for the inhibition of VEGF expression

In another aspect, the present invention relates to a pharmaceutical composition comprising a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents for the treatment of cancer.

In another aspect, the present invention relates to a pharmaceutical composition comprising a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents for the treatment of colon cancer.

In another aspect, the present invention relates to a pharmaceutical composition comprising a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents for the treatment of colon cancer wherein the PDE5 inhibitor is preferably 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

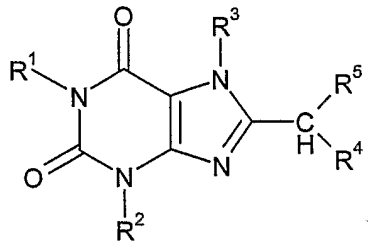
Detailed Description of the Preferred Embodiments

In one aspect of the present invention, there is provided a method of treating cancer comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal in need thereof. Warm-blooded animals includes, but is not limited to, mammals, especially humans.

Any PDE5 inhibitor may be employed within the scope of the present invention. Representative PDE5 inhibitors include, but are not limited to, sildenafil citrate, marketed under the tradename VIAGRA®; tadalafil, marketed under the tradename CIALIS® and disclosed in U.S. Patent No. 6,143,746; vardenafil, marketed under the tradename LEVITRA® and disclosed in WO 99/24433; UK-357903 owned by Pfizer; E8010E-4010 from

Eisai; LAS34179 owned by Almiral; FR-229934 co-owned by TAP/Fujisawa; EMD221829 co-owned by Merck/Lipha and T-1790 co-owned by Vivus/Tanabe.

Preferably, the PDE5 inhibitors of the present invention are compounds of formula (I)



(I)

in free or salt form, where

R^1 is hydrogen or alkyl optionally substituted by hydroxy, alkoxy or alkylthio;

R^2 is hydrogen, alkyl, hydroxyalkyl, alkylcarbonyloxyalkyl, alkoxyalkyl, alkylthioalkyl, alkenyl, cycloalkylalkyl, heterocyclylalkyl, aralkyl in which the aryl ring thereof is optionally fused to a 5-membered heterocyclic group or is optionally substituted by one or more substituents selected from alkoxy, amino, alkylamino, dialkylamino, acylamino, halogen, hydroxy, aminosulfonyl, alkylaminosulfonyl, dialkylaminosulfonyl, alkylsulfonylamino or dialkylaminosulfonylamino;

R^3 is hydrogen or alkyl optionally substituted by hydroxy, alkoxy or alkylthio;

R^4 is hydrogen or alkyl;

R^5 is a quinolinyl, isoquinolinyl or oxodihydroisoquinolinyl group optionally fused to a 5-membered heterocyclic group and optionally substituted by one or more substituents selected from halogen, cyano, hydroxy, alkyl, hydroxyalkyl, alkoxyalkyl, alkylthioalkyl, alkoxy, alkylthio, alkenyl, alkoxycarbonyl, alkynyl, carboxyl, acyl, a group of formula $-N(R^6)R^7$, aryl optionally substituted by one or more substituents selected from halogen or alkoxy, or heteroaryl having 5 or 6 ring atoms, attached through a ring carbon atom to the indicated carbon atom; and

R^6 and R^7 are each, independently, hydrogen or alkyl optionally substituted by hydroxy or alkoxy, or

one of R^6 and R^7 is hydrogen and the other is acyl, or

R^6 and R^7 together with the nitrogen atom to which they are attached denote a 5- or 6-membered heterocyclyl group.

"Alkyl", as used herein, denotes straight chain or branched alkyl, which may be, e.g., C_1-C_{10}-alkyl, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, straight- or branched-pentyl, straight- or branched-hexyl, straight- or branched-heptyl, straight- or branched-octyl, straight- or branched-nonyl or straight- or branched-decyl. Preferably alkyl is C_1-C_8-alkyl.

"Alkoxy", as used herein, denotes straight-chain- or branched-alkoxy which may be, e.g., C_1-C_{10}-alkoxy, such as methoxy, ethoxy, *n*-propoxy, isopropoxy, *n*-butoxy, isobutoxy, *sec*-butoxy, *tert*-butoxy, straight- or branched-pentoxy, straight- or branched-hexyloxy, straight- or branched-heptyloxy, straight- or branched-octyloxy, straight- or branched-nonyloxy or straight- or branched-decyloxy. Preferably, alkoxy is C_1-C_4-alkoxy.

"Alkylthio", as used herein, may be C_1-C_{10}-alkylthio, such as methylthio, ethylthio, *n*-propylthio, isopropylthio, *n*-butylthio, *sec*-butylthio, isobutylthio, *tert*-butylthio, pentylthio, hexylthio, heptylthio, octylthio, nonylthio or decylthio. Preferably alkylthio is C_1-C_4-alkylthio.

"Alkenyl", as used herein, means straight-chain- or branched-alkenyl, which may be, e.g., C_2-C_{10}-alkenyl, such as vinyl, 1-propenyl, 2-propenyl, 1-butenyl, isobutenyl or straight- or branched-pentenyl, -hexenyl, -heptenyl, -octenyl, -nonenyl or -decenyl. Preferred alkenyl is C_2-C_4-alkenyl.

"Cycloalkylalkyl", as used herein, denotes alkyl, e.g., C_1-C_{10}-alkyl, such as one of the C_1-C_{10}-alkyl groups hereinbefore mentioned, substituted by a C_3-C_8-cycloalkyl group, such as cyclopropyl, methylcyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, methylcyclohexyl, dimethylcyclohexyl, cycloheptyl or cyclooctyl. Preferably, cycloalkylalkyl is C_3-C_6-cycloalkyl-C_1-C_4-alkyl.

"Heterocyclylalkyl", as used herein, denotes alkyl, e.g., C_1-C_{10}-alkyl, such as one of the C_1-C_{10}-alkyl groups hereinbefore mentioned, substituted by a 5- or 6-membered heterocyclyl group having one or two hetero atoms selected from nitrogen, oxygen and sulfur in the ring, such as pyrrolyl, pyrrolidinyl, furyl, thienyl, pyridyl, piperidyl, imidazolyl, imidazolidinyl, pyrazolidinyl, piperazinyl, morpholinyl, oxazolyl or furazanyl. Preferably, heterocyclylalkyl is C_1-C_4-alkyl substituted by a 5- or 6-membered heterocyclyl group having one or two nitrogen or oxygen atoms or one nitrogen atom and one oxygen atom in the ring.

"Aralkyl", as used herein, means C_6-C_{10}-aryl-C_1-C_{10} alkyl and may be, e.g., one of the C_1-C_{10}-alkyl groups mentioned hereinbefore, particularly one of the C_1-C_4-alkyl groups,

substituted by phenyl, tolyl, xylyl or naphthyl. Preferably, aralkyl is phenyl-C_1-C_4-alkyl, particularly benzyl or 2-phenylethyl.

"Acyl", as used herein, denotes alkylcarbonyl, e.g., C_1-C_{10}-alkylcarbonyl, where C_1-C_{10}-alkyl may be one of the C_1-C_{10}-alkyl groups hereinbefore mentioned, optionally substituted by one or more halogen atoms; cycloalkylcarbonyl, e.g., C_3-C_8-cycloalkylcarbonyl, where C_3-C_8-cycloalkyl may be, e.g., cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl; 5- or 6-membered heterocyclylcarbonyl having one or two hetero atoms selected from nitrogen, oxygen and sulfur in the ring, such as furylcarbonyl or pyridylcarbonyl; arylcarbonyl, e.g., C_6-C_{10}-arylcarbonyl, such as benzoyl or aralkylcarbonyl, e.g., C_6-C_{10}-aryl-C_1-C_4-alkylcarbonyl, such as benzylcarbonyl or phenylethylcarbonyl. Preferably acyl is C_1-C_4-alkylcarbonyl.

"Alkynyl", as used herein, denotes straight or branched alkynyl, e.g., C_2-C_6-alkynyl, such as ethynyl, propargyl, 2-butynyl, pentynyl or hexynyl. Preferably alkynyl is C_2-C_4-alkynyl.

"Aryl", as used herein, denotes a monovalent carbocylic aromatic group, e.g., C_6-C_{10}-aryl, such as phenyl, phenyl substituted by one or more, e.g., one, two or three, C_1-C_4-alkyl groups, or naphthyl. Preferably aryl is phenyl.

"Heteroaryl having 5 or 6 ring atoms", as used herein, denotes a monovalent aromatic heterocyclic group having 5 or 6 ring atoms of which one, two or three are selected from nitrogen, oxygen and sulfur, such as pyrrolyl, furyl, thienyl, pyridyl, pyrazolyl, imidazolyl, triazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, dithiazolyl, trithiazolyl, furazanyl, pyrazinyl, pyrimidinyl or triazinyl.

In alkylamino, dialkylamino, acylamino, dialkylaminosulfonylamino, alkylcarbonyl, alkylcarbonyloxy, alkoxycarbonyl, hydroxyalkyl, alkylthioalkyl and alkoxyalkyl, the alkyl, acyl or alkoxy groups as appropriate have the meanings hereinbefore described.

"Halogen", as used herein, may be fluorine, chlorine, bromine or iodine; preferably it is fluorine, chlorine or bromine.

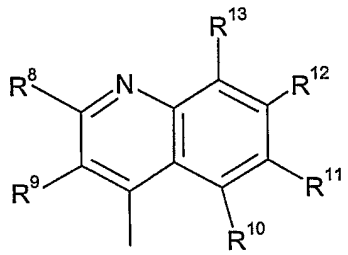
The 5-membered heterocyclic ring to which R^5 as a quinolinyl, isoquinolinyl or oxodihydroisoquinolinyl group is optionally fused may be, e.g., a 5-membered heterocyclic ring having one or two hetero atoms in the ring, said hetero atoms being selected from

oxygen, nitrogen and sulfur. Examples of such heterocyclic rings include pyrrole, pyrroline, pyrrolidine, furan, dihydrofuran, tetrahydrofuran, thiophene, dihydrothiophene, tetrahydrothiophene, imidazole, imidazoline, imidazolidine, pyrazole, pyrazoline, pyrazolidine, dioxolane, oxazole, isoxazole, thiazole and isothiazole rings. Preferably the 5-membered heterocyclic ring is a saturated ring having two hetero atoms, preferably two oxygen or two nitrogen atoms, especially two oxygen atoms.

R^5 as a quinolinyl group may be a 2-quinolinyl, 3-quinolinyl, 4-quinolinyl, 5-quinolinyl, 6-quinolinyl, 7-quinolinyl or 8-quinolinyl group, preferably a 4-quinolinyl, 5-quinolinyl or 8-quinolinyl group. R^5 as an isoquinolinyl group may be a 1-isoquinolinyl, 3-isoquinolinyl, 4-isoquinolinyl, 5-isoquinolinyl, 6-isoquinolinyl, 7-isoquinolinyl or 8-isoquinolinyl group, preferably a 1-isoquinolinyl or 4-isoquinolinyl group. In most of the especially preferred embodiments of the invention, R^5 is a 4-isoquinolinyl group.

R^5 as a substituted quinolinyl or isoquinolinyl group is preferably substituted by one, two, three or four of the abovementioned substituents, especially one, two or three of those substituents. The preferred substituted 4-isoquinolinyl group is preferably substituted in the 1- and/or 6- and/or 7- and/or 8-position of the isoquinoline ring system.

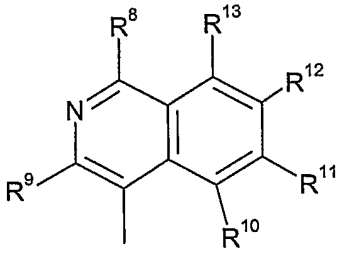
In especially preferred embodiments of the invention, R^5 is a quinolinyl group of formula (II)



(II)

,

or an isoquinolinyl group of formula



(III)

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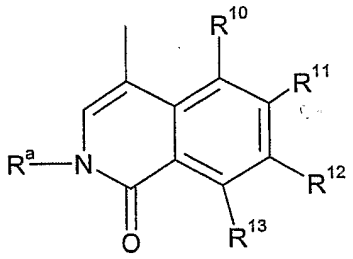
where

R^8, R^9, R^{10}, R^{11}, R^{12} and R^{13} are each, independently, hydrogen or a substituent selected from halogen, cyano, hydroxy, alkyl, hydroxyalkyl, alkoxyalkyl, alkylthioalkyl, alkoxy, alkylthio, alkenyl, alkoxycarbonyl, alkynyl, carboxyl, acyl, a group of formula -$N(R^6)R^7$, aryl optionally substituted by one or more substituents selected from halogen or alkoxy, or heteroaryl having 5 or 6 ring atoms, or

R^{11} and R^{12}, together with the carbon atoms to which they are attached, denote a 5-membered heterocyclic group having two oxygen or nitrogen atoms in the ring; and

R^6 and R^7 are as hereinbefore defined.

R^5 as an oxodihydroisoquinolinyl group preferably has the oxo group ortho to the ring nitrogen atom, preferably in the 1-position in the isoquinoline ring system. It is preferably linked to the remainder of the molecule of formula (I) via the ring carbon atom meta to the ring nitrogen atom, i.e., the 4-position in the isoquinoline ring system. An especially preferred oxodihydroisoquinolinyl group is of formula (IIIA)



(IIIA)

where

R^{10}, R^{11}, R^{12} and R^{13} are as hereinbefore defined; and

R^a is hydrogen or C_1-C_4-alkyl.

Preferred among the compounds of formula (I), in free or salt form, are those where

R^1 is hydrogen or C_1-C_4-alkyl optionally substituted by hydroxy, C_1-C_4-alkoxy or C_1-C_4-alkylthio;

R^2 is hydrogen, C_1-C_8-alkyl, hydroxy-C_1-C_8-alkyl, C_1-C_4-alkylcarbonyloxy-C_1-C_8-alkyl, C_1-C_4-alkoxy-C_1-C_8-alkyl, C_1-C_4-alkylthio-C_1-C_8-alkyl, C_2-C_4-alkenyl, C_3-C_8-cycloalkyl-C_1-C_4-alkyl, heterocyclyl-C_1-C_4-alkyl where the heterocyclyl group is a 5- or 6-membered heterocyclyl group having one or two hetero atoms selected from nitrogen and oxygen atoms in the ring, phenyl-C_1-C_4-alkyl in which the phenyl ring is optionally substituted by one or more substituents selected from C_1-C_4-alkoxy, amino, C_1-C_4-alkylamino, di(C_1-C_4-alkyl)amino, C_1-C_4-alkylcarbonylamino, halogen,

C_1-C_4-alkylsulfonylamino, or di(C_1-C_4-alkyl)aminosulfonylamino, and is optionally fused to a 5-membered heterocyclic ring having two oxygen or two nitrogen atoms in the ring;

R^3 is hydrogen or C_1-C_4-alkyl optionally substituted by hydroxy, C_1-C_4-alkoxy or C_1-C_4-alkylthio;

R^4 is hydrogen or C_1-C_4-alkyl;

R^5 is a quinolinyl, isoquinolinyl or oxodihydroisoquinolinyl group optionally fused to a 5-membered heterocyclic group having two oxygen or two nitrogen atoms in the ring and optionally substituted by one or more substituents selected from halogen, cyano, carboxy hydroxy, C_1-C_4-alkyl, hydroxy-C_1-C_4-alkyl, C_1-C_4-alkoxy-C_1-C_4-alkyl, C_1-C_4-alkylthio-C_1-C_4-alkyl, C_1-C_4-alkoxy, C_1-C_4-alkylthio, C_2-C_4-alkenyl, C_2-C_4-alkynyl, C_1-C_4-alkylcarbonyl, a group -N(R^6)R^7 or phenyl optionally substituted by one or more substituents selected from halogen or C_1-C_4-alkoxy; and

R^6 and R^7 are each, independently, hydrogen or C_1-C_4-alkyl optionally substituted by hydroxy or alkoxy, or

one of R^6 and R^7 is hydrogen and the other is C_1-C_4-alkylcarbonyl, or

R^6 and R^7, together with the nitrogen atom to which they are attached, denote a 5- or 6-membered heterocyclyl group having one or two nitrogen atoms and, optionally, an oxygen atom in the ring.

Further preferred among the compounds of formula (I) are those where

R^1 is hydrogen or C_1-C_4-alkyl;

R^2 is hydrogen, C_1-C_8-alkyl, hydroxy-C_1-C_8-alkyl, or C_1-C_4-alkylcarbonyloxy-C_1-C_8-alkyl, C_2-C_4-alkenyl, C_3-C_6-cycloalkyl-C_1-C_4-alkyl, heterocyclyl-C_1-C_4-alkyl where the heterocyclyl group is a 5-membered heterocyclyl group having one nitrogen or oxygen atom in the ring, phenyl-C_1-C_4-alkyl in which the phenyl ring is optionally substituted by one or two substituents selected from C_1-C_4-alkoxy, amino, C_1-C_4-alkylcarbonylamino, chlorine, bromine, C_1-C_4-alkylsulfonylamino, or di(C_1-C_4-alkyl)aminosulfonylamino and is optionally fused to a 5-membered heterocyclic ring having two oxygen atoms in the ring;

R^3 is hydrogen or C_1-C_4-alkyl;

R^4 is hydrogen or C_1-C_4-alkyl;

R^5 is a quinolinyl group of formula (II), an isoquinolinyl group of formula (III) or an oxodihydroisoquinolinyl group of formula (IIIA),

where

R^8, R^9, R^{10}, R^{11}, R^{12} and R^{13} are each, independently, selected from hydrogen, halogen, cyano, carboxy, hydroxy, C_1-C_4-alkyl, hydroxy-C_1-C_4-alkyl, C_1-C_4-alkoxy-C_1-C_4-alkyl, C_1-C_4-alkylthioC_1-C_4-alkyl, C_1-C_4-alkoxy, C_1-C_4-alkylthio, C_2-C_4-alkenyl, C_2-C_4-alkynyl, C_1-C_4-alkylcarbonyl, a group -N(R^6)R^7 or phenyl optionally substituted by one or two substituents selected from halogen or C_1-C_4-alkoxy, or

R^{11} and R^{12}, together with the carbon atoms to which they are attached, denote a 5-membered heterocyclic group having two oxygen atoms in the ring; and

R^6 and R^7 are each, independently, hydrogen or C_1-C_4-alkyl optionally substituted by hydroxy or alkoxy, or

one of R^6 and R^7 is hydrogen and the other is C_1-C_4-alkylcarbonyl, or

R^6 and R^7, together with the nitrogen atom to which they are attached, denote a 6-membered heterocyclyl group having one or two nitrogen atoms, or one nitrogen atom and one oxygen atom, in the ring.

Amongst the further preferred compounds hereinbefore described, especially preferred compounds are usually those in which R^5 is an isoquinolinyl group of formula (III), in which

R^8 is hydrogen, C_1-C_4-alkyl, halogen, cyano, -N(R^6)R^7,

where

R^6 and R^7 are, independently, C_1-C_4-alkyl, or

R^6 and R^7, together with the nitrogen atom to which they are attached, denote a 6-membered heterocyclyl group having one or two nitrogen atoms, or one nitrogen atom and one oxygen atom, in the ring, or phenyl substituted by one or two C_1-C_4-alkoxy groups;

R^9 and R^{10} are each, independently, hydrogen, C_1-C_4-alkyl or halogen;

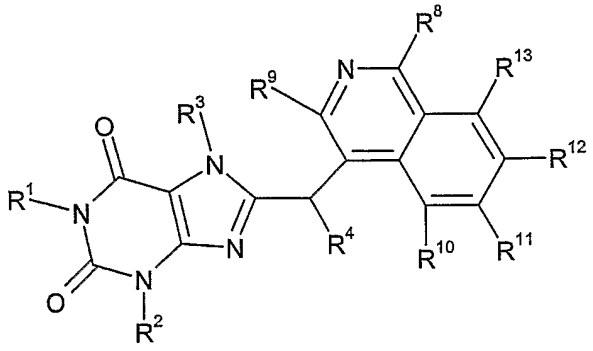
R^{11} and R^{12} are each, independently, hydrogen, halogen, cyano, carboxy, hydroxy, C_1-C_4-alkyl, C_1-C_4-alkoxy or C_2-C_4-alkynyl, or

R^{11} and R^{12}, together with the carbon atoms to which they are attached, denote a 5-membered heterocycle having two oxygen atoms in the ring; and

R^{13} is hydrogen or halogen.

Specific especially preferred compounds of formula (I) are those hereinafter described. More preferred amongst these are Compound Nos. 7, 10, 15, 35, 45, 49, 55, 60, 68 and 70.

Compounds of formula (I) which are also of formula (XXXXVI)



(XXXXVI)

where

R^1-R^4 and R^8-R^{13} are as hereinbefore defined, in free or salt form;

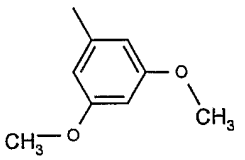
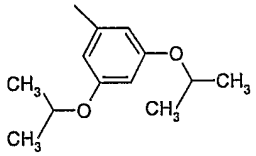
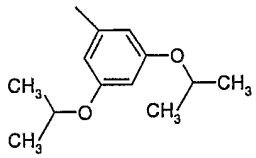
R^3 is H in all compounds except No. 44, where it is CH_3;

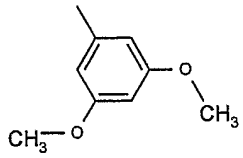
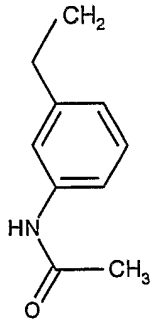
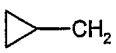
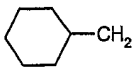
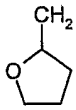
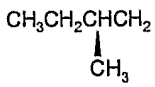
R^4 is H in all compounds except Nos. 25-27 and 41-43, where it is CH_3;

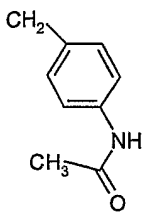
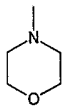
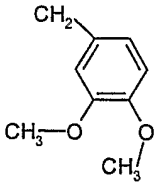
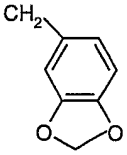
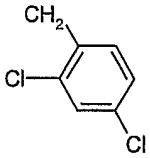
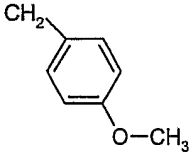
R^9 is H in all compounds except No. 29, where it is CH_3;

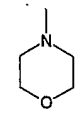
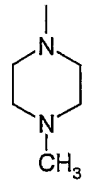
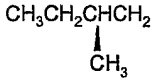
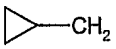
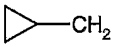
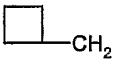
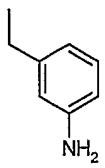
R^{10} is H in all compounds except No. 57, where it is Br and No. 75 where it is Cl; and

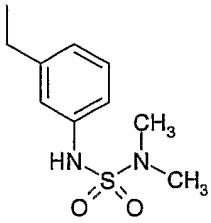
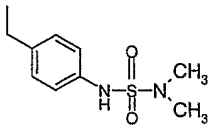
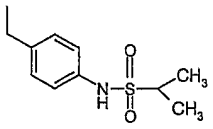
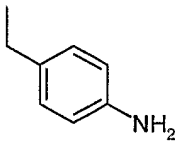
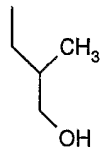
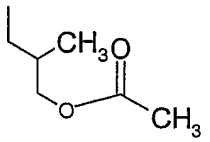
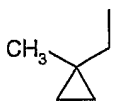
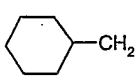
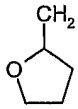
R^{13} is H in all compounds except No. 56 where it is F, and Nos. 65 and 66, where it is Br.

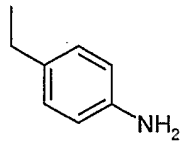
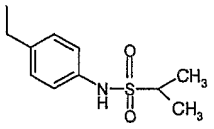
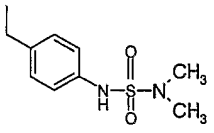
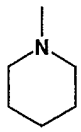
Ex. No.	R^1	R^2	R^8	R^{11}	R^{12}
1	CH_3	$(CH_3)_2CHCH_2$		OCH_3	OCH_3
2	CH_3	$(CH_3)_2CHCH_2$		OCH_3	OCH_3
3	CH_3	$(CH_3)_2CHCH_2$		$OCH(CH_3)_2$	OCH_3

Ex. No.	R^1	R^2	R^8	R^{11}	R^{12}
4	CH_3	$(CH_3)_2CHCH_2$		$OCH(CH_3)_2$	OCH_3
5	CH_3	$(CH_3)_2CHCH_2$	$(CH_3)_3C$	$OCH(CH_3)_2$	OCH_3
6	CH_3	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$	$OCH(CH_3)_2$	OCH_3
7	CH_3	$(CH_3)_2CHCH_2$	CH_3	OCH_3	OCH_3
8	CH_3	$(CH_3)_2CHCH_2$	$(CH_3)_3C$	OCH_3	OCH_3
9	CH_3	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$	OCH_3	OCH_3
10	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	OCH_3
11	CH_3		H	OCH_3	OCH_3
12	H	CH_3	H	OCH_3	OCH_3
13	CH_3	$CH_2=CHCH_2$	H	OCH_3	OCH_3
14	CH_3		H	OCH_3	OCH_3
15	CH_3	$(CH_3)_3CCH_2$	H	OCH_3	OCH_3
16	$(CH_3)_2CHCH_2$	$(CH_3)_2CHCH_2$	H	OCH_3	OCH_3
17	CH_3		H	OCH_3	OCH_3
18	CH_3	$CH_2=C(CH_3)CH_2$	H	OCH_3	OCH_3
19	CH_3		H	OCH_3	OCH_3
20	CH_3		H	OCH_3	OCH_3
21	H	$CH_3CH_2CH_2$	H	OCH_3	OCH_3

Ex. No.	R^1	R^2	R^8	R^{11}	R^{12}
22	CH_3		H	OCH_3	OCH_3
23	CH_3	$(CH_3)_2CHCH_2$	H		
24	CH_3	$(CH_3)_2CHCH_2$	H	H	OCH_3
25	CH_3	$(CH_3)_2CHCH_2$	Cl	H	OCH_3
26	CH_3	$(CH_3)_2CHCH_2$	CN	H	OCH_3
27	CH_3	$(CH_3)_2CHCH_2$		H	OCH_3
28	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	OH
29	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	OCH_3
30	CH_3	$CH_3(CH_2)_5$	H	OCH_3	OCH_3
31	CH_3		H	OCH_3	OCH_3
32	CH_3		H	OCH_3	OCH_3
33	CH_3		H	OCH_3	OCH_3
34	CH_3		H	OCH_3	OCH_3
35	CH_3	$(CH_3)_2CHCH_2$	Cl	OCH_3	OCH_3
36	CH_3	$(CH_3)_2CHCH_2$	H	H	H
37	CH_3	$(CH_3)_2CHCH_2$	H	OCH_2CH_3	OCH_3

Ex. No.	R^1	R^2	R^8	R^{11}	R^{12}
38	CH_3	$(CH_3)_2CHCH_2$		OCH_3	OCH_3
39	CH_3	$(CH_3)_2CHCH_2$		OCH_3	OCH_3
40	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	OCH_2CH_3
41	CH_3	$(CH_3)_2CHCH_2$	H	OCH_2CH_3	OCH_3
42	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	OCH_2CH_3
43	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	OCH_3
44	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	OCH_3
45	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	H
46	CH_3		H	OCH_3	H
47	CH_3	$(CH_3)_3CCH_2$	H	Cl	H
48	CH_3	$(CH_3)_2CHCH_2$	H	Cl	H
49	CH_3		H	OCH_3	H
50	CH_3		H	Cl	H
51	CH_3		H	OCH_3	OCH_3
52	CH_3	$CH_2=C(CH_3)CH_2$	H	OCH_3	H
53	CH_3	$(CH_3)_2CHCH_2$	H	Br	H
54	CH_3	$(CH_3)_3CCH_2$	H	OCH_3	H
55	CH_3	$(CH_3)_2CHCH_2$	H	C≡CH	H
56	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	H
57	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	H
58	CH_3		H	OCH_3	OCH_3

Ex. No.	R^1	R^2	R^8	R^{11}	R^{12}
59	CH_3		H	OCH_3	OCH_3
60	CH_3		H	OCH_3	OCH_3
61	CH_3		H	OCH_3	OCH_3
62	CH_3		H	OCH_3	OCH_3
63	CH_3	$(CH_3)_2CHCH_2$	H	H	OH
64	CH_3	$(CH_3)_2CHCH_2$	H	OH	OH
65	CH_3	$(CH_3)_2CHCH_2$	H	OH	OH
66	CH_3	$(CH_3)_2CHCH_2$	H	H	OH
67	CH_3	$HO(CH_2)_3$	H	OCH_3	OCH_3
68	CH_3		H	OCH_3	OCH_3
69	CH_3		H	OCH_3	OCH_3
70	CH_3		H	OCH_3	OCH_3
71	CH_3		H	OCH_3	H
72	CH_3		H	OCH_3	H

Ex. No.	R^1	R^2	R^8	R^{11}	R^{12}
73	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	F
74	CH_3	$(CH_3)_2CHCH_2$	H	CO_2H	H
75	CH_3	$(CH_3)_2CHCH_2$	H	OCH_3	H
76	CH_3	$(CH_3)_2CHCH_2$	H	CN	H
77	CH_3	$(CH_3)_2CHCH_2$	H	CH_2CH_3	H
78	CH_3	$(CH_3)_2CHCH_2$	H	OCH_2CH_3	H
79	CH_3		H	OCH_3	H
80	CH_3		H	OCH_3	H
81	CH_3		H	OCH_3	H
82	CH_3	$(CH_3)_2CHCH_2$	$N(CH_2)_3$	OCH_3	OCH_3
83	CH_3	$(CH_3)_2CHCH_2$		OCH_3	OCH_3
83	CH_3	$(CH_3)_2CHCH_2$	CH_3	OCH_3	H
84	CH_3	$(CH_3)_2CHCH_2$	CH_3	$OCH(CH_3)_2$	H
85	CH_3	$(CH_3)_2CHCH_2$	CH_3	OCH_2CH_3	H

Most preferred is Compound No. 45, which is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

Compounds of formula (I) may be in the form of salts, particularly pharmaceutically acceptable salts. Pharmaceutically acceptable acid addition salts of compounds of formula (I) include those of inorganic acids, e.g., hydrohalic acids, such as hydrofluoric acid, hydrochloric acid, hydrobromic acid or hydroiodic acid, nitric acid, sulfuric acid, phosphoric acid; and organic acids, e.g., aliphatic monocarboxylic acids, such as formic acid, acetic acid, trifluoroacetic acid, propionic acid and butyric acid; aliphatic hydroxy acids, such as lactic acid, citric acid, tartaric acid or malic acid; dicarboxylic acids, such as maleic acid or succinic acid; aromatic carboxylic acids, such as benzoic acid, *p*-chlorobenzoic acid,

diphenylacetic acid or triphenylacetic acid; aromatic hydroxy acids, such as *o*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, 1-hydroxynaphthalene-2-carboxylic acid or 3-hydroxynaphthalene-2-carboxylic acid; and sulfonic acids, such as methanesulfonic acid or benzenesulfonic acid. Pharmaceutically acceptable base salts of compounds of formula (I), where R^3 is hydrogen include metal salts, particularly alkali metal or alkaline earth metal salts, such as sodium, potassium, magnesium or calcium salts; and salts with ammonia or pharmaceutically acceptable organic amines or heterocylic bases, such as ethanolamines, benzylamines or pyridine. These salts may be prepared from free compounds of formula (I) or other salts of compounds of formula (I) by known salt-forming procedures.

Compounds of formula (I), in free form, may be converted into salt form, and vice versa, in a conventional manner. The compounds in free or salt form can be obtained in the form of hydrates or solvates containing a solvent used for crystallization. The compounds of formula (I), in free or salt form, can be recovered from reaction mixtures in a conventional manner. Isomer mixtures can be separated into individual isomers, e.g., enantiomers, in a conventional manner, e.g., by fractional crystallization.

The above compounds can be prepared as per the disclosure of WO 01/77110, incorporated by reference herein in its entirety as if set forth in full herein.

The inhibiting properties of the PDE5 inhibitors of the invention may be demonstrated in the cell culture and proliferation assay set forth in Example 1.

Illustrative of the invention, Compound No. 45 has an IC_{50} value of 2.0 μM in SW480 cells, whereas sildenafil was inactive in all tested cell lines. Moreover, this compound significantly repressed VEGF expression.

Having regard to these properties, the compounds of the invention may be employed in the treatment of conditions mediated by expression of VEGF. Treatment may be symptomatic or prophylactic.

Accordingly, the compounds of the invention may be employed in the treatment of conditions related to abnormal cellular proliferation, e.g., cancer.

Compounds of the invention are of particular interest for employment in the treatment of colon cancer.

In accordance therewith, the present invention relates to the treatment of a condition mediated by expression of VEGF, e.g., a disease related to abnormal cellular proliferation, such as cancer, in particular, colon cancer, comprising administration of a therapeutically effective amount of a pharmaceutical composition comprising a PDE5 inhibitor to a warm-blooded animal, especially a human, in need thereof.

The compounds of the present invention may be employed, e.g., in the preparation of pharmaceutical compositions that comprise an effective amount of the active ingredient together or in admixture with a significant amount of inorganic or organic, solid or liquid, pharmaceutically acceptable carriers.

The pharmaceutical compositions according to the invention are compositions for enteral, such as nasal, rectal or oral; or parenteral, such as intramuscular or intravenous, administration to warm-blooded animals (human beings and animals) that comprise an effective dose of the pharmacological active ingredient alone or together with a significant amount of a pharmaceutically acceptable carrier. The dose of the active ingredient depends on the species of warm-blooded animal, body weight, age and individual condition, individual pharmacokinetic data, the disease to be treated and the mode of administration.

The pharmaceutical compositions comprise from approximately 1% to approximately 95%, preferably from approximately 20% to approximately 90%, active ingredient. Pharmaceutical compositions according to the invention may be, e.g., in unit dose form, such as in the form of ampoules, vials, suppositories, dragees, tablets or capsules.

The pharmaceutical compositions of the present invention are prepared in a manner known *per se*, e.g., by means of conventional dissolving, lyophilising, mixing, granulating or confectioning processes.

Solutions of the active ingredient, and also suspensions, and especially isotonic aqueous solutions or suspensions, are preferably used, it being possible, e.g., in the case of lyophilised compositions that comprise the active ingredient alone or together with a carrier, e.g., mannitol, for such solutions or suspensions to be made up prior to use. The pharmaceutical compositions may be sterilised and/or may comprise excipients, e.g., preservatives, stabilisers, wetting agents and/or emulsifiers, solubilisers, salts for regulating the osmotic pressure and/or buffers, and are prepared in a manner known *per se*, e.g., by means of conventional dissolving or lyophilising processes. The said solutions or

suspensions may comprise viscosity-increasing substances, such as sodium carboxymethylcellulose, carboxymethylcellulose, dextran, polyvinylpyrrolidone or gelatin.

Suspensions in oil comprise as the oil component the vegetable, synthetic or semi-synthetic oils customary for injection purposes. There may be mentioned as such especially liquid fatty acid esters that contain as the acid component a long-chained fatty acid having from 8-22 carbon atoms, especially from 12-22 carbon atoms, e.g., lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid or corresponding unsaturated acids, e.g., oleic acid, elaidic acid, erucic acid, brassidic acid or linoleic acid, if desired with the addition of antioxidants, e.g., vitamin E, β-carotene or 3,5-di-*tert*-butyl-4-hydroxytoluene. The alcohol component of those fatty acid esters has a maximum of 6 carbon atoms and is a mono- or poly-hydric, e.g., a mono-, di- or tri-hydric, alcohol, e.g., methanol, ethanol, propanol, butanol or pentanol or the isomers thereof, but especially glycol and glycerol. The following examples of fatty acid esters are therefore to be mentioned: ethyl oleate, isopropyl myristate, isopropyl palmitate, "Labrafil M 2375" (polyoxyethylene glycerol trioleate, Gattefosse, Paris), "Miglyol 812" (triglyceride of saturated fatty acids with a chain length of C_8-C_{12}, Chemische Werke Witten/Ruhr, Germany), but especially vegetable oils, such as cottonseed oil, almond oil, olive oil, castor oil, sesame oil, soybean oil and more especially groundnut oil.

The injection compositions are prepared in customary manner under sterile conditions; the same applies also to introducing the compositions into ampoules or vials and sealing the containers.

Pharmaceutical compositions for oral administration, which is preferred, can be obtained by combining the active ingredient with solid carriers, if desired granulating a resulting mixture, and processing the mixture, if desired or necessary, after the addition of appropriate excipients, into tablets, dragee cores or capsules. They can also be incorporated into plastics carriers that allow the active ingredients to diffuse or be released in measured amounts.

Suitable carriers are especially fillers, such as sugars, e.g., lactose, saccharose, mannitol or sorbitol; cellulose preparations and/or calcium phosphates, e.g., tri-calcium phosphate or calcium hydrogen phosphate; and also binders, such as starch pastes using, e.g., corn, wheat, rice or potato starch, gelatin, tragacanth, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone;

and/or, if desired, disintegrators, such as the above-mentioned starches, also carboxymethyl starch, crosslinked polyvinylpyrrolidone, agar, alginic acid or a salt thereof, such as sodium alginate. Excipients are especially flow conditioners and lubricants, e.g., silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate; and/or polyethylene glycol. Dragee cores are provided with suitable, optionally enteric, coatings, there being used, *inter alia*, concentrated sugar solutions which may comprise gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents, or, for the preparation of enteric coatings, solutions of suitable cellulose preparations, such as ethylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Capsules are dry-filled capsules made of gelatin and also soft, sealed capsules made of gelatin and a plasticiser, such as glycerol or sorbitol. The dry-filled capsules may comprise the active ingredient in the form of granules, e.g., with fillers, such as lactose; binders, such as starches; and/or glidants, such as talc or magnesium stearate, and if desired with stabilisers. In soft capsules, the active ingredient is preferably dissolved or suspended in suitable oily excipients, such as fatty oils, paraffin oil or liquid polyethylene glycols, it likewise being possible for stabilisers and/or antibacterial agents to be added. Dyes or pigments may be added to the tablets or dragee coatings or to the capsule casings, e.g., for identification purposes or to indicate different doses of active ingredient.

These pharmaceutical preparations are for oral administration to warm-blooded animals, with the preparations comprising the pharmacological active compound either alone or together with customary pharmaceutical auxiliary substances. For example, the pharmaceutical preparations consist of from about 0.1-90%, preferably of from about 1% about 80%, of the active compound. These are prepared in a manner that is known *per se*, e.g., using conventional mixing, granulation, coating, solubulizing or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compound with solid excipients, if desired, granulating a mixture which has been obtained, and, if required or necessary, processing the mixture or granulate into tablets or coated tablet cores after having added suitable auxiliary substances.

A unit dosage for a mammal of about 50-70 kg may contain between about 1 mg and 1000 mg, advantageously between about 5-500 mg of the active ingredient, even more advantageously between about 5 mg to about 360 mg, more preferably, between about 5 mg to about 100 mg and even more preferably, between about 5 mg to about 50 mg. A therapeutically effective dosage of active compound is dependent on the species of warm-

blooded animal, the body weight, age and individual condition, on the form of administration, and on the compound involved, however, the term "therapeutically effective amount" is meant to include any dosage of a compound of the invention, within the above stated range which acts therapeutically, to prevent, treat or ameliorate the conditions mentioned herein.

The compounds of the present invention may also be used in combination with other PDE5 inhibitors or with other therapeutic agents suitable for the treatment of cancer, particularly colon cancer.

The following Example serves to illustrate and not limit the invention.

EXAMPLE 1 Cell culture and proliferation assay

Materials and methods

SW480 human colorectal adenocarcinoma cells, HCT116 human colon carcinoma cells, WI-38 human normal lung fibroblasts were obtained from ATCC (Rockville, ND). PRSC human prostate stroma cells were provided by G. Hampton. The cells were cultured at 5% CO_2 and 37°C in the following media: SW480; RPMI 1640 medium, 10% FBS, 2mM *L*-glutamine, HCT116; Mac Coy's 5A, 10% FBS, 4 mM *L*-glutamine, WI-38; DMEM with 4.5 g/L glucose, 10% FBS, 2 mM *L*-glutamine, 0.1 mM NEAA, 1% MEM vitamins and PRSC cells, DMEM with 4.5 g/L glucose, 10% FBS, 2 mM *L*-glutamine. Cells were seeded at a density of 2.5 x 10^4/well/50 µL culture medium in 96-well culture plates. The plates were incubated for 24 hours at 37°C, 5% CO_2. Ten (10) mM DMSO stock solutions of the tested compounds were diluted in culture medium to obtain final concentrations of 0.01, 0.03, 01, 0.3, 1, 3, 10 and 30 µM. Fifty (50) µL of the diluted solutions were added to the 96-well culture plates. 0.3% DMSO final concentration was added to positive control. One hundred (100) µL of culture medium was included to serve as blank. Forty-eight (48) hours after compound addition, the rate of cell proliferation was determined using the CellTiter96™ MTT assay (Promega), following the manufacturer's protocol. The absorbance at 550 nm was recorded using an ELISA plate reader (Dynatech MR5000). IC_{50} concentrations in µM were calculated.

Cyclic GMP Enzyme Immunoassay (EIA)

Cells were seeded at a density of 2 x 10^6/10 mL culture medium in 100 mm culture dishes. The cells were incubated for 24 hours at 37°C, 5% CO_2. Ten (10) mM DMSO stock solutions of Compound No. 45 and sildenafil were diluted in culture medium to obtain final

concentrations of 3, 6 and 12 µM. The culture medium was replaced with medium containing compound or 0.12% DMSO. Cells were incubated for 24 or 48 hours at 37°C, 5% CO_2. Cells were harvested by trypsinization and washed once with PBS. Cell numbers were evaluated and cGMP was extracted from cell pellets by addition of ice cold 65% ethanol, following the Biotrak cGMP EIA protocol 2 (Amersham Pharmacia Biotech). The extracts were dried in a speed vacuum, dissolved in assay buffer and cGMP levels were analyzed as described in protocol 2. The absorbance at 450 nm was recorded using an ELISA plate reader (Dynatech MRX). Results were calculated in fmol per million cells.

Treatment of stable transfected SW480 cells with Compound No. 45

The three SW480 cells were stable transfected with the reporter plasmids STOP, SRFOP or STAL. The reporter plasmid STOP contains TCF binding sites followed by TATA-like promoter (pTAL) region from HSV-TK promoter and Luciferase, the reporter plasmid SRFOP contains mutated Tcf binding sites followed by pTAL and Luciferase and the reporter plasmid STAL, contains pTAL promoter and Luciferase only.

Stably transfected SW480 cells were plated in a 96-well plate at a density of 20,000 cells/well and incubated over night at 37°C, 5% CO_2. One (1) µL compound was added to a final concentration of 10 µM Compound No. 45 or 0.1% DMSO. Twenty-four (24) hours after stimulation, the cells were assayed for luciferase using BrightGlo reagent (Promega). Luciferase activity (CPS) per well was calculated.

cDNA microarray hybridization

Twenty (20) million SW480 or HCT116 cells at about 80% confluency were incubated for 24 or 48 hours in culture medium containing 3 or 30 µM Compound No. 45 or 0.3% DMSO. Total RNA from cell pellet was isolated, using the RNeasy kit (Qiagen). The quality of the obtained mRNA was tested using the RNA 6000 LabChip Kit of Agilent Technologies. RNA samples were labeled and hybridized to oligonucleotide arrays U95Av2 (Affymetrix). Labeling of samples, hybridization to oligonucleotide arrays and scanning were performed as described by Wodicka et al. (1997). Scanned image files were visually inspected for artifacts and analyzed with GENECHIP 3.1 (Affymetrix). Each image was then scaled to an average hybridization intensity of 200, which corresponds to ~3-5 transcripts per cell. See Wodicka et al. (1997), *supra*. The results were analyzed using GeneData Expressionist, see homepage of Genedata Inc. for details (www.genedata.com).

Real time reverse transcriptase polymerase chain reaction (real time RT-PCR)

One (1) million SW480, HCT116 or PRSC cells at about 80% confluency were incubated for 24, 48 or 72 hours in culture medium containing 3 or 30 µM Compound No. 45 or 0.3% DMSO. Total RNA from cell pellet was isolated, using the RNeasy kit (Qiagen). The quality of the obtained mRNA was tested using the RNA 6000 LabChip Kit of Agilent Technologies. The primers and probes listed in Table 1 were designed from mRNA sequences within the coding region or in the 3' untranslated region using the primer express software from PE Applied Biosystems. For real time RT-PCR, RNA concentrations between 1 and 50 ng/reaction were used to obtain signals between 15-35 cycles. The RT-PCR reactions were performed in a two-step system. cDNA was synthesised from 1 µg RNA using the Taqman reverse transcription reagents (Applied Biosystems), as described in the manufacturer's protocol. For each RNA preparation RT reactions were performed plus and minus RT to exclude samples with genomic DNA contamination. To normalise for variability in the initial concentration and quality of the total RNA, GAPDH was used as an endogenous reference in all quantitation experiments. PCR reactions were performed using the Taqman Reaction System (Eurogentec). PCR reactions were mixed and thermal cycled as described in the manufacturer's protocol. Triplicate measurements were done for target and for reference reactions. The data were analysed using the comparative CT method for quantification as recommended by the manufacturer.

Cell treatment for hypoxia induction

SW480 cells were washed twice with serum-free medium (SFM) plus 0.1% BSA and seeded at a density of 5 x 10^5/5 mL medium in 60 mm culture dishes. The cells were incubated for 24 hours at 37°C, 5% CO_2. Ten (10) mM DMSO stock solution of Compound No. 45 was diluted in SFM plus 0.1% BSA to obtain final concentrations of 1.5 µM, 100 mM $CoCl_2$ stock solution was diluted in SFM plus 0.1% BSA to obtain final concentrations of 6.25 or 12.5 µM. The medium was replaced with SFM plus 0.1% BSA containing Compound No. 45 or 0.06% DMSO, plus or minus PMA or $CoCl_2$. Cells were incubated for 48 hours at 37°C, 5% CO_2. Cell supernatants were analyzed for VEGF levels and cell pellets were lysed for immunoblotting.

VEGF enzyme linked immuno assay (ELISA)

Cell supernatants were removed, microcentrifuged for 5 minutes at 12000 rpm. Supernatants were diluted 1:2 with calibrator diluent RD5K and VEGF ELISA was performed as described by the manufacturer (Quantikine # DVE00, R&D Systems). The absorbance at 450 nm was recorded using an ELISA plate reader (Dynatech MRX) Cells were lysed for immunoblotting as described below and protein concentrations were determined. VEGF levels were calculated in pg/mL/mg protein.

Cell culture treatment for Immunoblotting

SW480 and HCT116 cells were seeded at a density of 3 x 10^5/5 mL culture medium in 60 mm culture dishes. The cells were incubated for 24 hours at 37°C, 5% CO_2. A 10 mM DMSO stock solution of NVP-QAD17 was diluted in culture medium to obtain final concentrations of 1.5, 3, 6 and 12 µM. The culture medium was replaced with medium containing compound or 0.12% DMSO. Cells were incubated for 24 or 72 hours at 37°C, 5% CO_2. Cells were lysed for immunoblotting.

Cell lysis and Immunoblotting

SW480 and HCT116 cells were rinsed with PBS and lysed with 300 µL lysis buffer (50 mM Tris-HCl pH 7.5, 1% SDS). To shear genomic DNA, the cell lysates were processed in a FastPrep instrument for 20 seconds at a speed rating of 4, using the green FastRNA tubes (Bio101). Protein concentrations were determined according to the PIERCE-method (BCA Protein Assay, Reagent A and B). Protein concentration of lysates was adjusted to 1 µg/µL with lysis buffer. Four (4) parts of cell lysates were mixed with 1 part of 5-fold concentrated Laemmli buffer. A quantity of 10 µg (EphA2) or 20 µg (p21, GADD45A, HIF1α) total protein per lane was separated by 10-20% (p21, GADD45A) or 4-12% (EphA2, HIF1α) PAGEr Gold precast gel (BMA) on Mini Protean III (Bio-Rad) and transferred to PVDF membranes (Immomilon-P, Millipore) by a wet transfer system (Mini Trans-blot, Bio-Rad). Membranes were blocked with 5% milk powder in 0.05% Tween-TBS, incubated with specific antibodies anti-p21/CIP (1:1000, Transduction Laboratories), anti-GADD45A (1:1000, Santa Cruz), anti-EphA2 (1:2000, Upstate Biotechnology) or anti-HIF1α (1:500,Transduction Laboratories) mouse monoclonal antibodies, together with β-tubulin mouse monoclonal antibody (1:5000, Sigma) and the secondary anti-mouse HRP conjugated antibody (1:3000, BioRad) diluted in 5% milk powder/Tween-TBS. Detection of the target proteins on the

membranes was performed using the *SuperSignal*® chemiluminescent-substrate (PIERCE) on X-ray film (CL-XPosure, PIERCE).

Plasmid construction encoding small hairpin RNA

Two oligonucleotides, covering all four isoforms of PDE5A were ordered from GenScan (GenScan BipChip Technologies GmbH, Freiburg, Germany).

PDE5 sense oligonucleotide 5'-ATTAAGCTTTCCCGGAACAGATGCCTCTAA CC TTCAAGAGAGGTTAGAGGCATCTGTTCCTTTTTCTCGAGGCC-3' and PDE5 anti-sense oligonucleotide: 5'-GGCCTCGAGAAAAAGGAACAGATGC CTCTAACC TCTCTTGAAGGTTAGAGGCATCTGTTCCGGGAAAGCTTAAT-3'

The sense and anti-sense oligonucleotides were annealed in a thermal cycler, from 95°C to 24°C, 1°C per 5 minutes. The expression vectors pBH1 and pBH1-Hygro (kindly provided by C. Zimmermann, Novartis Pharma AG, Switzerland) contain an expression cassette suitable for intracellular expression of small hairpin siRNA. The transcription of siRNA is driven by a H1 polymerase III promoter and is terminated at position 2 of a 4-5-thymidine transcription termination site. The vectors pBH1 and pBH1-Hygro and the annealed oligo-nucleotides were cut first with *XHo*I and then with *Hind*III. The *Xho*I-*Hind*III digested vectors were treated with alkaline phosphatase and ligated with the PDE5/*Xho*I-*Hind*III oligonucleotides.

Stable transfection of pBH1-Hygro::PDE5 siRNA plasmid

SW480 cells were seeded at a density of 5 x 10^5/2 mL culture medium in 6-well plates (35 mm). Cells were transfected with 3 µL Lipofectamine 2000 (Invitrogen) and 1.0 µg vector or pBH1-Hygro::PDE5 siRNA plasmid, following the manufacturer's protocol. Five (5) hours after DNA-liposome complex addition, transfected cells were supplemented with 1 mL culture medium containing 20% FBS. Cells were passaged to a 100 mm culture dish, 24 hours after transfection. The growth medium was replaced by growth medium containing 0.4 mg/mL hygromycin B (Invitrogen). The medium plus hygromycin B was replaced twice a week. Cells were passage 6-10 days after hygromycin B addition. Cells from passage 3 were seeded at a density of 1 x 10^3/well/100 µL culture medium in 96-well culture plates. The plates were incubated for 0, 1, 2 and 3 days at 37°C, 5% CO_2. The rate of cell proliferation was determined as described the section cell culture and proliferation assay.

Cells from passage 5 or 9 were washed once with PBS and lysed with 600 µL lysis buffer (Qiagen, RNeasy Mini Kit) RNA was isolation and real time RT-PCR experiments were performed as described earlier.

Although the present invention has been described in considerable detail with reference to certain preferred versions thereof, other versions are possible without departing from the spirit and scope of the preferred versions contained herein. All references referred to herein are hereby incorporated by reference in their entirety.

What is claimed is

1. A method of inhibiting angiogenesis comprising administering a therapeutically effective amount of a phosphodiesterase 5 (PDE5) inhibitor to a warm-blooded animal in need thereof.

2. A method of inhibiting vascularization comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal in need thereof.

3. A method of inhibiting vascular endothelial growth factor (VEGF) expression comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal in need thereof.

4. The method of Claim 3, wherein the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

5. A method treating cancer comprising administering a therapeutically effective amount of a PDE5 inhibitor to a warm-blooded animal in need thereof.

6. The method of Claim 5, wherein the cancer is colon cancer.

7. The method of Claim 6, wherein the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

8. The method of Claim 7, wherein the warm-blooded animal is a human.

9. A method of inhibiting VEGF expression comprising administering a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents to a warm-blooded animal in need thereof.

10. The method of Claim 9, wherein the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

11. The method of Claim 10, wherein the warm-blooded animal is a human.

12. A method of treating cancer comprising administering a therapeutically effective amount of a PDE5 inhibitor in combination with one or more additional therapeutic agents to a warm-blooded animal in need thereof.

13. The method of Claim 12, wherein the PDE5 inhibitor is 3-isobutyl-8-(6-methoxy-isoquinolin-4-ylmethyl)-1-methyl-3,7-dihydro-purine-2,6-dione.

14. The method of Claim 13, wherein the warm-blooded animal is a human.

15. The method of Claim 14, wherein the cancer is colon cancer.

INTERNATIONAL SEARCH REPORT

Interna Application No
PCT/EP2005/001377

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/522 A61P35/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THOMPSON W J ET AL: "Exisulind induction of apoptosis involves guanosine 3',5'-cyclic monophosphate phosphodiesterase inhibition, protein kinase G activation, and attenuated 'beta!-catenin" CANCER RESEARCH 01 JUL 2000 UNITED STATES, vol. 60, no. 13, 1 July 2000 (2000-07-01), pages 3338-3342, XP002336691 ISSN: 0008-5472	5,6,8,12
Y	the whole document	5,7,8, 12-14

-/--

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2005	01/08/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Collura, A

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Interr — Application No
PCT/EP2005/001377

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 01/77110 A (NOVARTIS AG; NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H; BHALA) 18 October 2001 (2001-10-18) cited in the application page 25, paragraphs 2,3 claims	5,7,8, 12-14
A		1-4,6, 9-11,15
A	WO 03/028730 A (NOVARTIS AG; NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H; COHEN) 10 April 2003 (2003-04-10) claims	1-15

INTERNATIONAL SEARCH REPORT

ıal application No.
. .T/EP2005/001377

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-15
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Intern Application No
PCT/EP2005/001377

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