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☐ Patente de Modelo de Utilidad

☒ Capítulo I

☐ Capítulo II

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ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☐ Poderes, si fuere el caso.
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- ☐ De ser el caso, modificaciones efectuadas a la solicitud internacional, Nuevas Reivindicaciones
- ☒ Otros: Informe de Búsqueda de Antecedentes Internacional junto con la traducción
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FIGURA CARACTERISTICA

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Solicito la concesión de la patente,

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From the INTERNATIONAL BUREAU

PCTNOTIFICATION CONCERNING
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To:

BAYER HEALTHCARE AG
Law and Patents
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Applicant BAYER HEALTHCARE AG et al	

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Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
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Certificate

Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr.

Patent application No.

Demande de brevet n°

05021261.2 / EP05021261

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP05021261

Der Präsident des Europäischen Patentamts;
Im Auftrag
For the President of the European Patent Office
Le Président de l'Office européen des brevets
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Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

PDE inhibitors and combinations thereof for the treatment of urological disorders

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PDE inhibitors and combinations thereof for the treatment of urological disorders**Technical field of the invention**

The present invention relates to phosphodiesterases (PDEs) and the pharmacology of PDE inhibitors. More particularly, the invention relates to PDE-5 and PDE-4 inhibitors and their use for preparation of medicaments for the treatment of urological disorders.

Background of the invention

Benign prostate hyperplasia (BPH) resulting in bladder outlet obstruction (BOO) is a very common neoplasm in men. It is estimated that approximately 80% of men older than 50 years have moderate to severe symptoms, including increased urinary frequency, nocturia and urgency, accompanied by a slow urinary stream and urinary retention. Therefore BPH is increasingly recognized as a major health care problem in westernized countries (Guess 1995). Besides prostatic surgery (20% of all BPH patients), the common treatment of the disease comprises 5-alpha reductase inhibitors (finasteride) and alpha blockers (tamsulosin, doxazosin, terazosin, alfuzosin) (Truss 2001). 5-alpha reductase inhibitors influence the mechanical component of BPH and inhibit proliferation of the prostate tissue. Alpha blockers influence the dynamic component and decrease the irritative symptoms of BPH via relaxation of the prostatic smooth muscle which decreases the urethral resistance. Moreover alpha-blockers are able to relax directly bladder smooth muscle cells and reduce the non voiding contractions of the bladder. However all these treatment options have limited efficacy and/or an unfavorable side effect profile (Carbone 2003). Thus, diverse attempts have focused on new therapeutic options to inhibit the proliferation of the prostatic stroma or to decrease the tone of the smooth muscle of the prostate and bladder. This includes i.e. aromatase inhibitors (Sciarrà 2000), growth factor antagonists (Desgrandchamps 1997), potassium channel openers (Gopalakrishnan 2004), and endothelin antagonists (Andersson 2002).

It is also well established that the cyclic nucleotides cAMP and cGMP can reduce smooth muscle tone (Drescher 1994). cAMP and cGMP are synthesized from their corresponding nucleoside triphosphates by the adenylate and guanylate cyclase respectively. They are degraded by the cyclic nucleotide phosphodiesterases (PDEs) which regulate the intracellular cAMP and cGMP level very effectively. Up to now 11 different PDE family members have been identified which differ in structure, regulation and specificity for their substrate (Soderling 2000). The role of PDEs for the treatment of Urological Disorders is only poorly understood, the characterization of PDE isoforms has lagged behind other systems and much of the literature was published prior to identification of the newly identified PDEs. Although PDEs are expressed in the lower urinary tract i.e. in bladder,

urethral and in prostate tissues, mRNA expression data and direct comparisons of all PDE isogenes are still missing or inconsistent. There are some evidences that unspecific PDE inhibition is able to relax human prostate tissue (Drescher 1994). The data about the effect of PDE-5 inhibition is very limited. It has been shown, that Zaprinast, a PDE-5 inhibitor which also inhibits PDE-6, -9 and -11 is able to relax pre-contracted human prostate tissue in vitro (Ückert 2001), whereas the role of other PDE families within this tissue needs to be determined. Within the bladder, unspecific blockade of different PDEs by IBMX (inhibition of PDE-1, -2, -3, -4, -5, -6, -10, and -11) could relax bladder of female Guinea Pigs whereas Zaprinast was ineffective (Gillespie 2004). Despite these inconsistent findings the role of PDE-5 in relaxation of the corpus cavernosum and the treatment of erectile dysfunction is well known and there are already very potent and selective PDE-5 inhibitors in the market. Potent and selective PDE-4 inhibitors are mainly used for the treatment of Asthma and COPD (Spina 2003).

Disclosure of the invention

One aspect of the invention is provided by a PDE mRNA expression profile demonstrating the abundance of cGMP-dependent PDE-5 and cAMP-dependent PDE-4 not only in the prostate but also in bladder tissue (Figures 1, 2). Therefore, selective inhibitors of PDE-5 and PDE-4, and in particular combinations of both, i. e. Vardenafil, Sildenafil or Tadalafil in combination with Roflumilast, should not only reduce prostate contractility but also, as an additional benefit, ameliorate irritative symptoms caused by bladder outlet obstruction as it frequently occurs in urological disorders. Urological disorders addressed by therapeutic agents of the invention comprise Benign Prostate Hyperplasia (BPH), Lower Urinary Tract Symptoms (LUTS) and in particular irritative symptoms caused by BPH-induced bladder outlet obstruction (BOO). Because not only symptomatic irritations of the bladder but also underlying BPH-induced bladder outlet obstructions are addressed by treatment with specific PDE-5 and PDE-4 inhibitors (and in particular combinations thereof), this treatment provides substantial advantage over methods of treatment already known in the art. Other disorders related to urology which may be treated by compounds of the invention are genitourinary disorders comprising benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies, neurogenic bladder syndrome, urinary incontinence like urge-, stress-, or overflow incontinence, pelvic pain, and erectile dysfunction.

Another aspect of the invention is the demonstration that the PDE-5 inhibitor Vardenafil has a relaxing effect on rat urethral rings with an EC₅₀ value of 0.96 µmol/l, and rat prostate and bladder

strips with the EC₅₀ value of 1.1 and 5.0 µmol/l respectively (Figure 3, Table 1).

Another aspect of the invention is the demonstration that the PDE-4 inhibitor Roflumilast and the PDE-5 inhibitor Vardenafil both show relaxing effects on rabbit bladder strips with an IC₅₀ of 260 nmol/l and 1.7 µmol/l respectively (Figure 4, Table 2).

- 5 Another aspect of the invention is provided by the demonstration that the PDE-5 inhibitor Vardenafil significantly decreased the number of non-voiding contractions as a measure of irritative symptoms of BPH in the rat bladder outlet obstruction (BOO) model (Figure 5).

The invention provides PDE-5 inhibitors which are, alone or in combination with PDE-4 inhibitors, useful for the treatment of urological disorders. In particular, compounds of the
 10 invention are Tadalafil ((6R,12aR) -2,3,6,7,12,12a - Hexahydro - 2 - methyl - 6 - (3,4-methylene-dioxyphenyl) pyrazino(1',2':1,6) pyrido(3,4-b)indole-1,4-dione), Vardenafil (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl)-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]- 7- methyl- 9- propyl-2,4,7,8-tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), Roflumilast (3-(cyclopropylmethoxy)-N-(3,5-
 15 dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxy-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid), and Piclamilast (3-cyclopentoxy-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide).

Still another aspect of the invention is a method of screening for PDE inhibitors, in particular for inhibitors of PDE-4- and PDE-5 for use, alone or in combination, for the preparation of
 20 medicaments for the treatment of urological disorders, i. e. Benign Prostate Hyperplasia (BPH) and in particular irritative symptoms caused by BPH-induced bladder outlet obstruction (BOO) including, but not limited to, Lower Urinary Tract Symptoms (LUTS).

The invention provides methods (also referred to herein as "screening assays") for identifying PDE inhibitors which can be used for the treatment of urological disorders. The methods entail the
 25 identification of candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other molecules) which bind to phosphodiesterases and/or have a stimulatory or inhibitory effect on the biological activity of PDE1A or its expression and then determining which of these compounds have an effect on symptoms or diseases regarding urological disorders in an in vivo assay.

30 Candidate or test compounds or agents which bind to PDE-4 or PDE-5 and/or have a stimulatory or inhibitory effect on the activity or the expression of PDE-4 or PDE-5 are identified either in assays that employ cells which express PDE-4 and/or PDE-5 (cell-based assays) or in assays with

isolated PDE-4 and/or PDE-5 (cell-free assays). The various assays can employ a variety of variants of PDEs (e. g., full-length PDEs, a biologically active fragment of PDEs, or a fusion protein which includes all or a portion of PDEs). Moreover, PDE-4 and/or PDE-5 can be derived from any suitable mammalian species. The assay can be a binding assay entailing direct or indirect measurement of the binding of a test compound or a known PDE-4 or PDE-5 ligand to PDE-4 or PDE-5. The assay can also be an activity assay entailing direct or indirect measurement of the activity of PDE-4 or PDE-5. The assay can also be an expression assay entailing direct or indirect measurement of the expression of PDE-4 and/or PDE-5 mRNA or PDE-4 and/or PDE-5 protein. The various screening assays are combined with an in vivo assay entailing measuring the effect of the test compound on the symptoms of urological disorders.

The present invention includes biochemical, cell free assays that allow the identification of inhibitors and agonists of PDEs suitable as lead structures for pharmacological drug development. Such assays involve contacting PDE-4 and/or PDE-5 with a test compound and determining the ability of the test compound to act as an antagonist (preferably) or an agonist of the enzymatic activity of PDE4 and/or PDE-5. In one embodiment, the assay includes monitoring the PDE activity of PDE-4 and/or PDE-5 by measuring the conversion of either cP or cGMP to its nucleoside monophosphate after contacting PDE-4 and/or PDE-5 with a test compound.

For example, cAMP and cGMP levels can be measured by the use of the tritium containing compounds 3HcAMP and 3HcGMP as described in [Hansen, R. S., and Beavo, J.A., PITAS USA1982,79: 2788-92]. To screen a compound pool comprised of a large number of compounds, the microtiter plate-based scintillation proximity assay (SPA) as described in [Bardelle, C. et al. (1999) Anal. Biochem. 275: 148-155] can be applied.

Alternatively, the phosphodiesterase activity of the recombinant protein can be assayed using a commercially available SPA kit (Amersham Pharmacia). The PDE enzyme hydrolyzes cyclic nucleotides, e.g. cAMP and cGMP to their linear counterparts. The SPA assay utilizes the tritiated cyclic nucleotides [3H]cAMP or [3H]cGMP, and is based upon the selective interaction of the tritiated non cyclic product with the SPA beads whereas the cyclic substrates are not effectively binding.

Radiolabelled product bound to the scintillation beads generates light that can be analyzed in a scintillation counter.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parcnteral e.g., intravenous, intradermal, subcutaneous' oral (e.g.' inhalation)' transdermal (topical) transmucosal and rectal

administration. Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyethylene glycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed.

Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or stearates; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For

transdermal administration; the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal
5 delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Bio degradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen,
10 polyorthoesters, and polylactic acid.

In another embodiment the invention provides combinations of PDE-4 and PDE-5 inhibitors and their use for the preparation of pharmaceutical compositions for the treatment of urological disorders, whereby these combinations comprise either i) pharmaceutical compositions comprising a compound having inhibitory action on both PDE-4 and PDE-5 activity, or ii) pharmaceutical
15 compositions comprising at least one PDE-4 inhibitor and at least one PDE-5 inhibitor as a fixed combination in one application unit, or iii) a kit of parts containing at least two sets of pharmaceutical compositions, each set consisting of at least one pharmaceutical preparation comprising a PDE-5 inhibitor in units of at least one dose and at least one pharmaceutical preparation comprising a PDE-4 inhibitor in units of at least one dose, whereby each application
20 unit of said pharmaceutical compositions is administered in combination, sequentially, as single dose or in multiple doses.

In particular, the present invention provides:

A method of screening for PDE 5 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH),
25 Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a test compound (which may or may not have PDE-4 inhibitory activity) with a PDE5 polypeptide, ii) determining the activity of the PDE5 polypeptide at a certain concentration of the test compound or in the absence of said test compound, iii) determining the activity of said PDE5 polypeptide at a different concentration of said test compound, iv) selecting
30 at least one compound with inhibitory effect on the PDE-5 polypeptide.

A method of screening for PDE 4 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH),

Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a test compound (which may or may not have PDE-5 inhibitory activity) with a PDE4 polypeptide, ii) determining the activity of the PDE4 polypeptide at a certain concentration of the test compound or in the absence of said test compound, iii) determining the activity of said PDE4 polypeptide at a different concentration of said test compound, iv) selecting at least one compound with inhibitory effect on the PDE-4 polypeptide.

A method of screening for combinations of PDE 5 inhibitors and PDE 4 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a first test compound with a PDE5 polypeptide, ii) determining the activity of the PDE5 polypeptide at a certain concentration of the first test compound or in the absence of said first test compound, iii) determining the activity of the PDE5 polypeptide at a different concentration of said first test compound, iv) selecting at least one first compound with inhibitory effect on the PDE-5 polypeptide, v) contacting a second test compound with a PDE4 polypeptide, vi) determining the activity of the PDE4 polypeptide at a certain concentration of the second test compound or in the absence of said second test compound, vii) determining the activity of the PDE4 polypeptide at a different concentration of said second test compound, vii) selecting at least one second compound with inhibitory effect on the PDE-4 polypeptide, vii) combining at least one first compound with PDE5 inhibitory activity with at least one second compound having PDE4 inhibitory activity.

Methods of screening which involve contacting the test compound in or at the surface of a cell, wherein the cell is in vitro.

Methods of screening which involve contacting the test compound with the PDE-4 or PDE-5 polypeptide in a cell free system.

25 Methods of screening may involve a test compound which is coupled to a detectable label.

A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which regulates the activity of a PDE5 polypeptide.

30 A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower

Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which regulates the activity of a PDE4 polypeptide.

A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower
5 Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which is a combination of the therapeutic agents of claims 10 and 11.

A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower
10 Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which regulates the activity of a PDE5 polypeptide and a PDE4 polypeptide.

A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower
Urinary Tract Symptoms (LUTS) in a mammal comprising a PDE-4 inhibitor selected from the
group of PDE-4 Inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy) -N-(3,5-
15 dichloropyridin-4-yl) - 4 -(difluoro-methoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxy-4-methoxy-phenyl) - cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxy-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide).

A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower
20 Urinary Tract Symptoms (LUTS) in a mammal comprising a PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of Vardenafil (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]- 7- methyl-1- 9- propyl-1,2,4,7,8- tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), and Tadalafil ((6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-
25 (3,4-methylenedioxyphenyl).

A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower
Urinary Tract Symptoms (LUTS) in a mammal comprising a combination of at least one PDE-4
inhibitor selected from the group of PDE-4 inhibitors consisting of Roflumilast (3-
30 (cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxy-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxy-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide) and at least one PDE-5 inhibitor selected from the group of PDE-5 inhibitors consisting of Vardenafil (2-(2-Ethoxy-5-(4-

ethylpiperazin-1-yl-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]- 7- methyl-1- 9- propyl-1,2,4,7,8-tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), and Tadalafil ((6R,12aR) -2,3,6,7, 12, 12a-Hexahydro - 2 - methyl - 6 - (3,4-methylene-dioxyphenyl)).

- 5 Use of a PDE5 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

Use of a PDE4 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH),

- 10 Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

Use of a combination of at least one PDE4 inhibitor and at least one PDE5 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

- 15 Use of an agent which is a inhibitor of a PDE4 polypeptide and a PDE5 polypeptide for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

Use of PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of Vardenafil (2-(2-Ethoxy-5- (4-ethylpiperazin-1-yl-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]- 7- methyl-1- 9- propyl-1,2,4,7,8- tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), and Tadalafil ((6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)) for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases

- 25 consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

Use of PDE-4 inhibitor selected from the group of PDE-4 Inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxo-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxo-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide) for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower

- 30

Urinary Tract Symptoms (LUTS) in a mammal.

Use of a combination of at least one PDE-4 inhibitor selected from the group of PDE-4 inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxo-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxo-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide) and at least one PDE-5 inhibitor selected from the group of PDE-5 inhibitors consisting of Vardenafil (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl)-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4) triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl] - 7-methyl-9-propyl-2,4,7,8-tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), and Tadalafil ((6R,12aR) -2,3,6,7,12,12a-Hexahydro -2-methyl-6-(3,4-methylene-dioxyphenyl) for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

A Method for the preparation of a pharmaceutical composition useful for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising the steps of i) identifying a inhibitor of PDE5 according to the method of screening described above ii) identifying a inhibitor of PDE4 according to the method of screening described above) determining whether said inhibitors ameliorate the symptoms of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal; and iii) combining at least one of said inhibitors with an acceptable pharmaceutical carrier.

A method for the preparation of a pharmaceutical composition wherein the inhibitor of PDE5 is a PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of Vardenafil (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl)-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]- 7-methyl-9-propyl-2,4,7,8-tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), and Tadalafil ((6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl).

A method for the preparation of a pharmaceutical composition wherein the inhibitor of PDE4 is a PDE-4 inhibitor selected from the group of PDE-4 Inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxo-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxo-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide).

Use of a pharmaceutical composition as mentioned above for the regulation of PDE activity in a mammal having a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS).

- 5 A kit of parts for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal including humans containing a combination of at least one pharmaceutical composition selected from the group of pharmaceutical compositions consisting of Vardenafil, Sildenafil and Tadalafil and at least one pharmaceutical composition selected from the
- 10 group of pharmaceutical compositions consisting of Roflumilast, Cilomilast and Piclamilast.

- A Method for the preparation of a kit of parts useful for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising the steps of i) selecting at least one pharmaceutical composition from the group of pharmaceutical compositions
- 15 consisting of Vardenafil, Sildenafil and Tadalafil ii) selecting at least one pharmaceutical composition from the group of pharmaceutical compositions consisting of Roflumilast, Cilomilast and Piclamilast, iii) combining at least two of said pharmaceutical compositions thereby creating said kit of parts.

- A kit of parts for the treatment of a disease comprised in a group of diseases consisting of Benign
- 20 Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal containing a combination of at least one therapeutic agent regulating the activity of a PDE-5 polypeptide and Tadalafil and at least one therapeutic agent regulating the activity of a PDE-4 polypeptide.

- A Method for the preparation of a kit of parts useful for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising the steps of i) selecting at least one pharmaceutical composition comprising a therapeutic agent regulating the activity of a PDE-5 polypeptide, ii) selecting at least one pharmaceutical composition comprising a therapeutic agent regulating the activity of a PDE-4 polypeptide, iii) combining at least two of said
- 25 pharmaceutical compositions thereby creating said kit of parts.
 - 30

Description of figures

Figure 1: Relative mRNA Expression of PDE-5 in Kindney (K), Bladder (B), Prostate (P), Urethra (U) and Corpus Cavemosum (C) of Sprague Dawley Rats. Data are mean + SEM, n=10.

5 Figure 2: Relative mRNA Expression of PDE-4a, -4b, -4c, -4d and PDE-5 in bladder and prostate of Sprague Dawley Rats. Data are mean + SEM, n=10.

10 Figure 3: Effects of Vardenafil on the contraction of isolated rat urethral rings (black triangles), and bladder- (black diamonds) and prostate strips (grey squares). The bladder strips were pre-contracted using K⁺ (50 mmol/l) Krebs-Henseleit solution. Prostate and urethral tissues were pre-contracted using 10 µmol/l Phenylephrine. The relaxation was expressed as percentage of the pre-contraction. Each point represents a mean value ± SEM. n = 9.

Figure 4: Effects of Roflumilast (black diamonds) and Vardenafil (grey squares) on the contraction of isolated rabbit bladder strips. The bladder strips were pre-contracted using K⁺ (50 mmol/l) Krebs-Henseleit solution. The relaxation was expressed as percentage of the pre-contraction. Each point represents a mean value ± SEM. n = 9.

15 Figure 5: Number of non-voiding contractions in % after bolus i.v. treatment with vehicle (V) and Vardenafil HCl (1, 3 and 10 mg/kg). Data are mean + SEM, * = significant with p<0.05 (paired student's t-test).

Example 1

20 Tissue sampling and RNA preparation: Male Sprague Dawley rats with a body weight between 200-250 g were used for tissue collecting. The rats were briefly anaesthetized with a mixture of 5% Isoflurane (Baxter S.A.) in a carrier with 70% N₂O and 30% O₂, and than euthanized by decapitation. The abdomen was opened by a midline incision and the kidneys and lower urinary tract tissue as renal medulla, urether, bladder prostate and urethra were exposed and quickly removed. The tissues were frozen in liquid N₂ and stored until RNA preparation. Total RNA was
25 isolated using RNeasy mini columns (Qiagen Inc.) and further purified by DNase digestion.

PDE mRNA quantification: The mRNA expression of the different PDE isogenes in rat lower urinary rat tissues was measured by real time quantitative PCR (TaqMan-PCR, Heid 1996). Therefore 1 µg of total RNA were transcribed into cDNA with Superscript II RT cDNA synthesis kit (Gibco, Inc) according to the manual of the supplier. The mRNA for the PDEs were measured
30 by real-time quantitative RT-PCR on an ABI Prism 7700 sequence detection instrument (Applied Biosystems, Inc.). Specific Sequences for forward and reverse primers as for the fluorogenic probe

of each PDE isogene mRNA were designed by Primer Express 1.5 Software (Applied Biosystems, Inc.). During PCR amplification, 5' nucleolytic activity of Taq polymerase cleaves the probe separating the 5' reporter fluorescent dye from the 3' quencher dye. The threshold cycle, Ct, which correlates inversely with the target mRNA level, was measured as the cycle number at which the reporter fluorescent emission increases 10 standard deviations above background level. As housekeeping gene, beta-actin was quantified as described above, using as forward primer 5'-accttcaacaccccagcca-3', reverse primer 5'-cagtggtagcaccagagga-3' and fluorescent probe 5'-6AFM-acgtagccatccaggctgtgtgtcc-TAMARA-3'. The PDE mRNA levels were corrected for beta-actin mRNA levels and calculated as relative expression using comparative Ct-method.

- 10 Expression of PDE-5 and PDE-4A, -4B, -4C, -4D mRNA in the lower urinary tract: Since there is only incomplete data on the expression profile of PDE-5 in lower urinary tract tissue, PDE mRNA was quantified in male Sprague Dawley rats via TaqMan RT-PCR. The most prominent expression of PDE-5 was found in the bladder (Figure 1). Lower expression levels were found in the urethra, the corpus cavernosum and the prostate (Figure 1). These result showed that there is a substantial
- 15 PDE-5 mRNA expression in lower urinary tract tissues especially in the bladder.

Moreover PDE-4 mRNA expression of all four PDE-4 isogenes (PDE-4A, -4B, -4C and -4D) was determined by TaqMan RT-PCR in the bladder and the prostate (Figure 2). We found very low expression of PDE-4c, which was on the borderline of detectability, however PDE-4A, -4B and -4D mRNAs were substantially expressed within both tissues. In the bladder PDE-4D was the most

20 abundant PDE-4 isogene mRNA whereas in the prostate PDE-4a and -4D are almost equally distributed and 2.5-fold higher expressed than PDE-5 mRNA (Figure 2).

The expression profile demonstrates that PDE-5 and PDE-4D mRNAs are abundant in the bladder but also in prostate tissue. Therefore, inhibitors of PDE-5 or PDE-4, but in particular combinations of both, PDE-5 and PDE-4 inhibitors, such as Vardenafil in combination with Roflumolast should

25 not only reduce bladder but also prostate contractility, thus providing advantage over methods of treatment for urological disorders already existing in the state of the art, said disorders comprising i. e. Benign Prostate Hyperplasia (BPH) and in particular irritative symptoms caused by BPH-induced bladder outlet obstruction (BOO) including, but not limited to, Lower Urinary Tract Symptoms (LUTS).

30 Example 2

Tissue preparation: Male Wistar rats (200-300 g) were euthanized using carbon dioxide. The tissues were removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl₂ 2.0 MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, glucose 11.5. Four

equally sized longitudinal strips of approximately 2 mm x 10 mm were cut from the bladder body. Prostate strips were obtained by cutting transversally through the lobes of the prostate gland parallel to the urethra. One ring per animal was dissected from the proximal part of the urethra.

- 5 White New Zealand rabbits were anesthetized using thiopental. The urinary bladder was removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl₂ 2.0 MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, and glucose 11.5. Equally sized longitudinal strips of approximately 2 mm x 10 mm were cut from the bladder body.

- 10 Recording of mechanical activity: The preparations were transferred to 20 ml organ baths containing Krebs-Henseleit solution equilibrated with 95% O₂, 5% CO₂ at 37°C. The strips were mounted between two hooks by means of two clips. For recording of isometric tension one of the hooks was connected to a force transducer which was in turn linked to an amplifier and chart recorder. The other hook was attached to a movable unit, permitting precise adjustment of preload tension. All tissues were then given a 60 min equilibration period during which they were washed and the resting tension was adjusted to 1 g every 20 min.

- 15 After the equilibration period, each experiment was started by exposing the preparation to K⁺ (50 mmol/l) Krebs-Henseleit solution. The procedure was repeated 3 times and the tissues were washed at least three times between each contraction.

- 20 The bladder strips were then pre-contracted using K⁺ (50 mmol/l) Krebs-Henseleit solution. When the contraction was stabilized, an accumulative dose response curve of the compound tested was constructed. The stabilized contraction induced by K⁺ (50 mmol/l) Krebs-Henseleit solution was defined as 100% tension. The relaxation was expressed as percentage tension.

Prostatic strips and urethral rings were pre-contracted using 10⁻⁶ mol/l phenylephrine. The effects of the compounds on the prostate tissue were tested in a non-cumulative manner with washing steps between each concentration.

- 25 Organ bath assay: effects of Vardenafil on isolated rat urogenital organs: The effects of the PDE5 inhibitor Vardenafil on the relaxation of smooth muscles were tested in the organ bath system. The compound was applied in the concentration range from 10⁻⁸ mol/l to 10⁻⁵ mol/l (Figure 3, Table 1). Vardenafil relaxed the urethral rings with an EC₅₀ value of 0.96 µmol/l, and the prostate and bladder strips with the EC₅₀ value of 1.1 and 5.0 µmol/l respectively.

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Concentration ($\mu\text{mol/l}$)	Bladder (% contraction)	Urethra (% contraction)	Prostate (% contraction)
0.001	94.3 $\pm$ 0.9	88.4 $\pm$ 2.4	-
0.01	91.2 $\pm$ 1.3	88.5 $\pm$ 2.1	-
0.1	89.9 $\pm$ 1.8	77.1 $\pm$ 3.9	99.2 $\pm$ 3.0
1	77.8 $\pm$ 2.1	44.9 $\pm$ 1.5	76.0 $\pm$ 2.3
10	25.3 $\pm$ 3.2	3.5 $\pm$ 1.7	25.3 $\pm$ 3.2

Table 1: Effects of vardenafil on the contraction of isolated rat urogenital tissues. The relaxation is expressed as percentage of the pre-contraction. Each point represents a mean value $\pm$ SEM. n = 9.

Example 3

- 5
- Tissue preparation: Male Wistar rats (200-300 g) were euthanized using carbon dioxide. The tissues were removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl₂ 2.0 MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, glucose 11.5. Four equally sized longitudinal strips of approximately 2 mm x 10 mm were cut from the bladder body. Prostate strips were obtained by cutting transversally through the lobes of the prostate gland parallel to the urethra. One ring per animal was dissected from the proximal part of the urethra.
- 10

White New Zealand rabbits were anesthetized using thiopental. The urinary bladder was removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl₂ 2.0 MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, and glucose 11.5. Equally sized longitudinal strips of approximately 2 mm x 10 mm were cut from the bladder body.

- 15
- Recording of mechanical activity: The preparations were transferred to 20 ml organ baths containing Krebs-Henseleit solution equilibrated with 95% O₂, 5% CO₂ at 37°C. The strips were mounted between two hooks by means of two clips. For recording of isometric tension one of the hooks was connected to a force transducer which was in turn linked to an amplifier and chart recorder. The other hook was attached to a movable unit, permitting precise adjustment of preload tension. All tissues were then given a 60 min equilibration period during which they were washed and the resting tension was adjusted to 1 g every 20 min.
- 20

After the equilibration period, each experiment was started by exposing the preparation to K⁺ (50 mmol/l) Krebs-Henseleit solution. The procedure was repeated 3 times and the tissues were washed at least three times between each contraction.

The bladder strips were than pre-contracted using K⁺ (50 mmol/l) Krebs-Henseleit solution. When the contraction was stabilized, an accumulative dose response curve of the compound tested was constructed. The stabilized contraction induced by K⁺ (50 mmol/l) Krebs-Henseleit solution was defined as 100% tension. The relaxation was expressed as percentage tension.

- 5 Prostatic strips and urethral rings were pre-contracted using 10⁻⁶ mol/l phenylephrine. The effects of the compounds on the prostate tissue were tested in a non-cumulative manner with washing steps between each concentration.

Effects of PDE5 and PDE4 inhibitors on isolated rabbit bladder strips: The effects of the PDE5 inhibitor vardenafil and the PDE4 inhibitor Roflumilast on the relaxation the bladder smooth
10 muscle was tested in the organ bath using rabbit bladder strips. Both compounds were tested in the concentration from 10⁻⁹ mol/l to 10⁻⁵ mol/l (Fig. 4, Tab. 2). Roflumilast and vardenafil both relaxed the bladder strips with an IC50 of 260 nmol/l and 1.7 µmol/l respectively. (Table 2)

Concentration (µmol/l)	Roflumilast (% contraction)	Vardcnafil (% contraction)
0.001	100.8 ± 0.6	100.9 ± 1.0
0.01	90.1 ± 1.7	99.9 ± 0.9
0.1	75.1 ± 2.1	95.7 ± 1.7
1	55.8 ± 2.5	76.3 ± 3.7
10	40.9 ± 3.3	9.6 ± 12.6

Table 2: Effects of Roflumilast and Vardenafil on the contraction of isolated rabbit bladder strips. The relaxation is expressed as percentage of the pre-contraction. Each point represents a mean
15 value ± SEM. n = 9.

Example 4

All animal experiments were performed due to the "German Law for the Protection of Laboratory animals" and were conducted due to the approved guidelines of the permission
20 "Tierversuchsvorhaben No 401/A01 M010/M011 vom 09.07.2004". Experiments were performed with female Sprague Dawley Rats with a body weight between 200-250g.

Bladder Outlet Obstruction: For the bladder outlet obstruction, rats were anesthetized with a mixture of 1.5-2% isoflurane in a carrier of 66% N₂O 33% O₂. The abdomen was shaved, opened by a lower midline incision, bladder and urethra were identified and the urethra-vesical junction was exposed. A 1.0 mm metal rod was placed along the proximal urethra and a 6-0 nylon ligature
25 was tied tightly around the urethra and the rod. The rod was consecutively removed and the

abdomen was closed by a silk ligature and cleaned up by 70% ethanol. There was a postoperative anti-pain treatment with 10 mg/kg Rimadyl® (Pfizer). Rats were kept then for 2 weeks and feed with tap water and standard rat chow. 24 hours prior to the cystometry rats were anaesthetized with isoflurane as described above. The laparotomy was performed as described above, the bladder was exposed and a polyethylene catheter (PE50) was implanted into the bladder dome. The catheter was tunneled subcutaneously using a cannula to reach the back neck of the animal. Additionally a catheter for intravenous administration (PE10) was placed into the jugular vein and tunneled subcutaneously to the back neck of the animal. Both catheters were fixed by a suture and a tape.

Conscious cystometry: For cystometry the animals were shortly anaesthetized by isoflurane as described above, placed in a Ballman's cage and fixed. Then animals were recovered at least for 1 h before the experiment started. The bladder catheter was then connected to the t-shaped tube to connect a pressure transducer for measurement of intra-bladder pressure (MLT0698, ADInstruments) and an infusion pump (Perfusor Compact®, Braun Melsungen) for continuous infusion of saline solution at a flow rate of 10 ml/h. The BOO animals showed an increase in bladder capacity (due to the bladder enlargement) and non voiding contractions (mimicking the irritative symptoms of BPH), when compared with a control animal. The efficacy of treatment was quantified via calculation of the non voiding contractions per micturition interval before and after treatment. For positive control the alpha receptor antagonist tamsulosin (10 µg/kg) was used. Values were given in % reduction of non voiding contractions.

Statistical analysis of results: Data are expressed as means ± standard error of the means (SEM), and *n* indicating the number of experiments. The significance of differences between means was determined by paired and unpaired Student's t-test. Probability levels less than 0.05 were considered significant.

Effect of vardenafil on non voiding contractions in BOO rats: For the BOO model a partial ligature of the urethra was performed in rat under anesthesia. The bladder outlet obstruction (BOO) resulting from this procedure caused a significant increase of bladder weight (data not shown) indicating a pronounced bladder hypertrophy. It also caused non voiding contractions (NVC) of the bladder which were detected via cystometry in conscious animals. These NVC were a measure of irritative symptoms in BPH.

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EPO - Munich
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29. Sep. 2005Claims

1. A method of screening for PDE 5 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a test compound with a PDE5 polypeptide, ii) determining the activity of the PDE5 polypeptide at a certain concentration of the test compound or in the absence of said test compound, iii) determining the activity of said PDE5 polypeptide at a different concentration of said test compound, iv) selecting at least one compound with inhibitory effect on the PDE-5 polypeptide.
2. A method of screening for PDE 4 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a test compound with a PDE4 polypeptide, ii) determining the activity of the PDE4 polypeptide at a certain concentration of the test compound or in the absence of said test compound, iii) determining the activity of said PDE4 polypeptide at a different concentration of said test compound, iv) selecting at least one compound with inhibitory effect on the PDE-4 polypeptide.
3. The method of claim 1 wherein the test compound is a PDE 4 inhibitor.
4. The method of claim 2 wherein the test compound is a PDE 5 inhibitor.
5. A method of screening for combinations of PDE 5 inhibitors and PDE 4 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a first test compound with a PDE5 polypeptide, ii) determining the activity of the PDE5 polypeptide at a certain concentration of the first test compound or in the absence of said first test compound, iii) determining the activity of the PDE5 polypeptide at a different concentration of said first test compound, iv) selecting at least one first compound with inhibitory effect on the PDE-5 polypeptide, v) contacting a second test compound with a PDE4 polypeptide, vi) determining the activity of the PDE4 polypeptide at a certain concentration of the second test compound or in the absence of said second test compound, vii) determining the activity of the PDE4 polypeptide at a different concentration of said second test compound, vii) selecting at least one second compound with inhibitory effect on the PDE-4 polypeptide, vii) combining at least one compound with PDE5 inhibitory activity with at least one compound having PDE4 inhibitory activity.

- 6. The method of any of claims 1 to 5, wherein the step of contacting is in or at the surface of a cell.
- 7. The method of any of claims 1 to 5, wherein the cell is in vitro.
- 8. The method of any of claims 1 to 5, wherein the step of contacting is in a cell free
5 system.
- 9. The method of any of claims 1 to 8, wherein the compound is coupled to a detectable label.
- 10. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and
10 Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which regulates the activity of a PDE5 polypeptide.
- 11. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which
15 regulates the activity of a PDE4 polypeptide.
- 12. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which is a combination of the therapeutic agents of claims 10 and 11.
- 20 13. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which regulates the activity of a PDE5 polypeptide and a PDE4 polypeptide.
- 25 14. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a PDE-4 inhibitor selected from the group of PDE-4 Inhibitors consisting of Roflumilast, Cilomilast and Piclamilast.
- 15. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and

30

Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of Vardenafil, Sildenafil and Tadalafil.

16. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a combination of at least one PDE-4 inhibitor selected from the group of PDE-4 inhibitors consisting of Roflumilast, Cilomilast and Piclamilast and at least one PDE-5 inhibitor selected from the group of PDE-5 inhibitors consisting of Vardenafil, Sildenafil and Tadalafil.

17. Use of a PDE5 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

18. Use of a PDE4 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

19. Use of a combination of at least one PDE4 inhibitor and at least one PDE5 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

20. Use of an agent which is a inhibitor of a PDE4 polypeptide and a PDE5 polypeptide for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

21. Use of PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of Vardenafil, Sildenafil and Tadalafil for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

22. Use of PDE-4 inhibitor selected from the group of PDE-4 Inhibitors consisting of Roflumilast, Cilomilast and Piclamilast for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia

(BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

23. Use of a combination of at least one PDE-4 inhibitor selected from the group of PDE-4 inhibitors consisting of Roflumilast, Cilomilast and Piclamilast and at least one PDE-5 inhibitor
5 selected from the group of PDE-5 inhibitors consisting of Vardenafil, Sildenafil and Tadalafil for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

24. A Method for the preparation of a pharmaceutical composition useful for the treatment
10 of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising the steps of i) identifying a inhibitor of PDE5 according to the method of claims 1 to 9, ii) identifying a inhibitor of PDE4 according to the method of claims 1 to 9, iii) determining whether said inhibitors ameliorate the symptoms of a disease comprised in a group of diseases
15 consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal; and iii) combining at least one of said inhibitors with an acceptable pharmaceutical carrier.

25. The method of claim 24 wherein the PDE-5 inhibitor is selected from the group of PDE-5 Inhibitors consisting of Vardenafil, Sildenafil and Tadalafil.

20 26. The method of claim 24 wherein the PDE-4 inhibitor is selected from the group of PDE-4 Inhibitors consisting of Roflumilast, Cilomilast and Piclamilast.

27. A kit of parts suitable for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal containing a combination of at least one
25 pharmaceutical composition of claim 14 and at least one pharmaceutical composition of claim 15.

28. A Method for the preparation of a kit of parts useful for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising the steps of i) selecting at least one of the pharmaceutical compositions of claim 14, ii) selecting at
30 least one of the pharmaceutical compositions of claim 15, iii) combining at least two of said pharmaceutical compositions thereby creating said kit of parts.

PDE inhibitors and combinations thereof for the treatment of urological disorders

Abstract

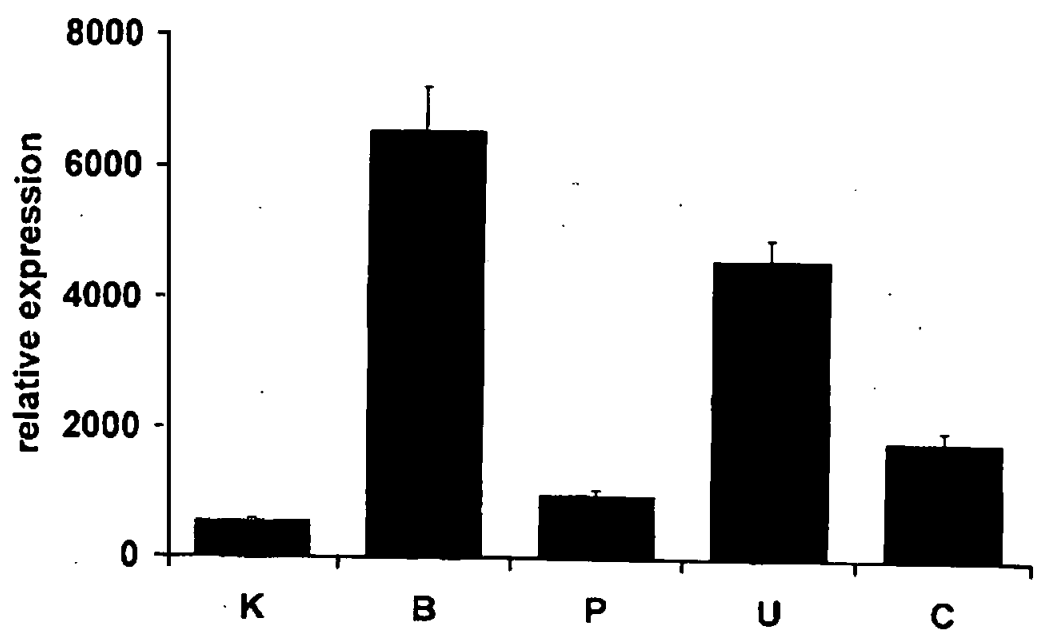
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29. Sep. 2005

The invention provides pharmacological compositions comprising PDE-5 and PDE-4 inhibitors, alone or in combination, for the treatment of urological disorders comprising Benign Prostate Hyperplasia (BPH), Lower Urinary Tract Symptoms (LUTS) and in particular irritative symptoms caused by BPH-induced bladder outlet obstruction (BOO). The invention also provides methods of screening for such PDE-5 and PDE-4 inhibitors for use, alone and in combination, in the preparation of medicaments for the treatment of said urological disorders.

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Fig. 1

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Fig. 2

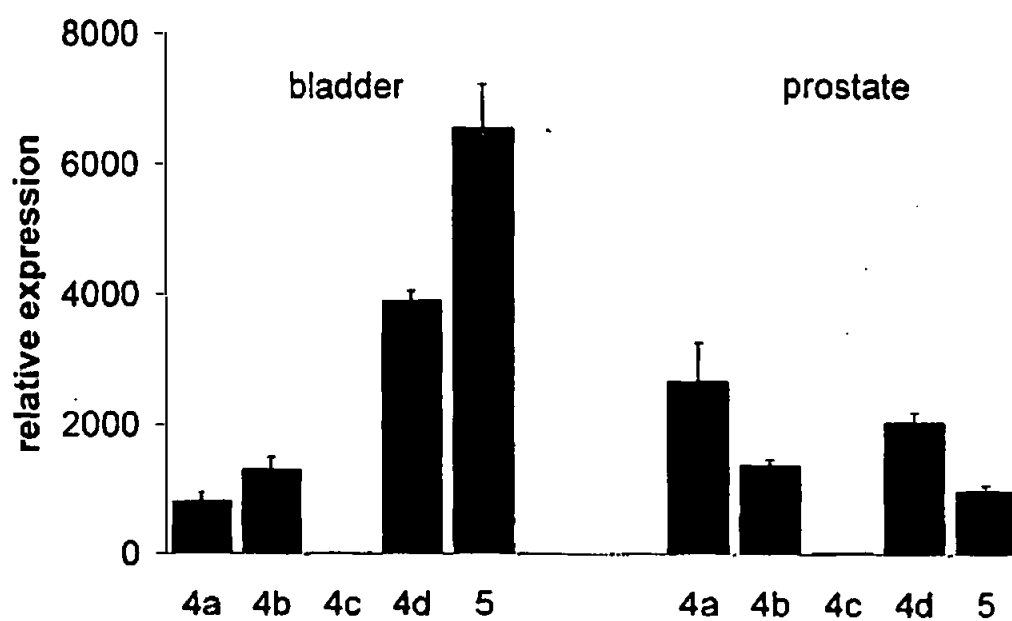


Fig. 3

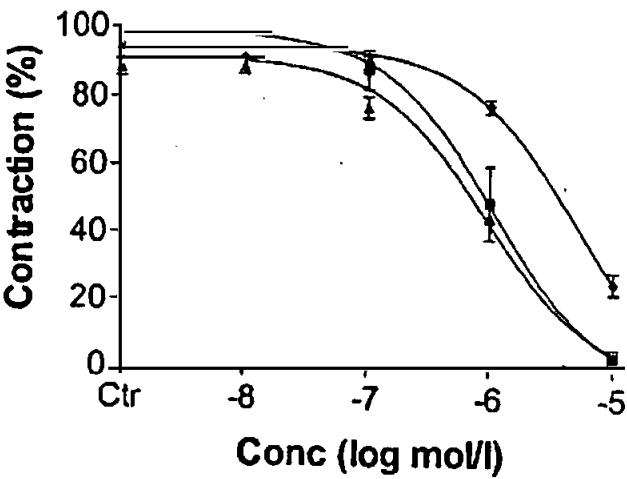
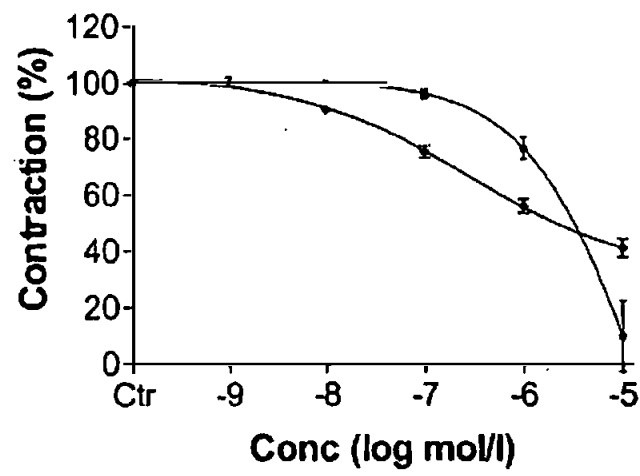
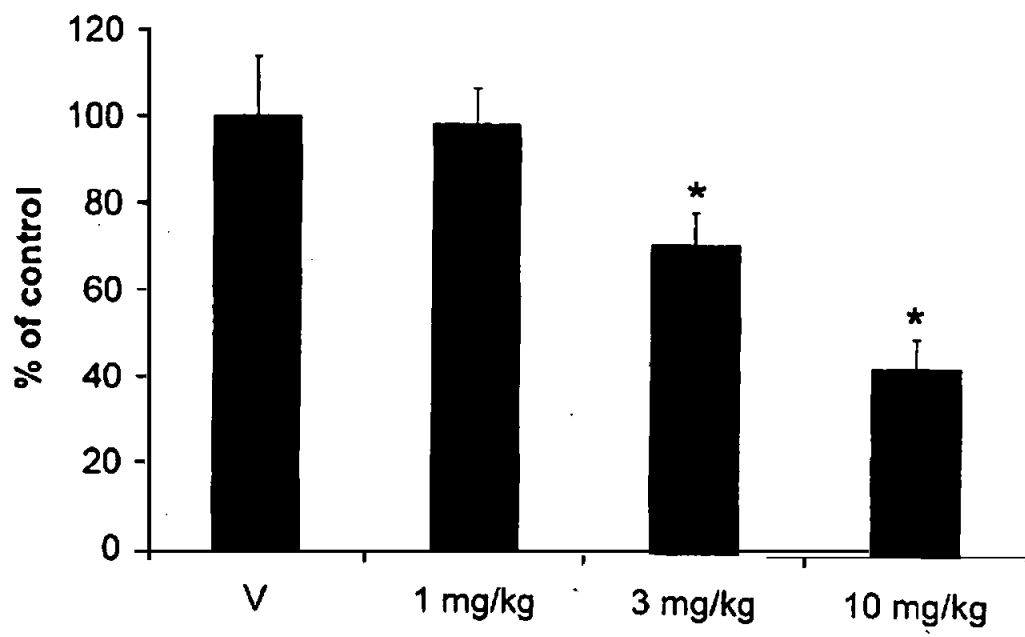


Fig.4



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Fig.5



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(54) Title: PDE INHIBITORS AND COMBINATIONS THEREOF FOR THE TREATMENT OF UROLOGICAL DISORDERS

(57) Abstract: The invention provides pharmacological compositions comprising PDE-5 and PDE-4 inhibitors, alone or in combination, for the treatment of urological disorders comprising Benign Prostate Hyperplasia (BPH), Lower Urinary Tract Symptoms (LUTS) and in particular irritative symptoms caused by BPH-induced bladder outlet obstruction (BOO). The invention also provides methods of screening for such PDE-5 and PDE-4 inhibitors for use, alone and in combination, in the preparation of medicaments for the treatment of said urological disorders.

PDE inhibitors and combinations thereof for the treatment of urological disorders**Technical field of the invention**

The present invention relates to phosphodiesterases (PDEs) and the pharmacology of PDE inhibitors. More particularly, the invention relates to PDE-5 and PDE-4 inhibitors and their
5 use for preparation of medicaments for the treatment of urological disorders.

Background of the invention

Benign prostate hyperplasia (BPH) resulting in bladder outlet obstruction (BOO) is a very common neoplasm in men. It is estimated that approximately 80% of men older than 50 years have moderate to severe symptoms, including increased urinary frequency, nocturia
10 and urgency, accompanied by a slow urinary stream and urinary retention. Therefore BPH is increasingly recognized as a major health care problem in westernized countries (Guess 1995). Besides prostatic surgery (20% of all BPH patients), the common treatment of the disease comprises 5-alpha reductase inhibitors (finasteride) and alpha blockers (tamsulosin, doxazosin, terazosin, alfuzosin) (Truss 2001). 5-alpha reductase inhibitors influence the
15 mechanical component of BPH and inhibit proliferation of the prostate tissue. Alpha blockers influence the dynamic component and decrease the irritative symptoms of BPH via relaxation of the prostatic smooth muscle which decreases the urethral resistance. Moreover alpha-blockers are able to relax directly bladder smooth muscle cells and reduce the non voiding contractions of the bladder. However all these treatment options have limited
20 efficacy and/or an unfavorable side effect profile (Carbone 2003). Thus, diverse attempts have focused on new therapeutic options to inhibit the proliferation of the prostatic stroma or to decrease the tone of the smooth muscle of the prostate and bladder. This includes i.e. aromatase inhibitors (Sciarra 2000), growth factor antagonists (Desgrandchamps 1997), potassium channel openers (Gopalakrishnan 2004), and endothelin antagonists (Andersson
25 2002).

It is also well established that the cyclic nucleotides cAMP and cGMP can reduce smooth muscle tone (Drescher 1994). cAMP and cGMP are synthesized from their corresponding nucleoside triphosphates by the adenylate and guanylate cyclase respectively. They are degraded by the cyclic nucleotide phosphodiesterases (PDEs) which regulate the
30 intracellular cAMP and cGMP level very effectively. Up to now 11 different PDE family members have been identified which differ in structure, regulation and specificity for their substrate (Soderling 2000). The role of PDEs for the treatment of Urological Disorders is

only poorly understood, the characterization of PDE isoforms has lagged behind other systems and much of the literature was published prior to identification of the newly identified PDEs. Although PDEs are expressed in the lower urinary tract i.e. in bladder, urethral and in prostate tissues, mRNA expression data and direct comparisons of all PDE isogenes are still missing or inconsistent. There are some evidences that unspecific PDE inhibition is able to relax human prostate tissue (Drescher 1994). The data about the effect of PDE-5 inhibition is very limited. It has been shown, that Zaprinast, a PDE-5 inhibitor which also inhibits PDE-6, -9 and -11 is able to relax pre-contracted human prostate tissue in vitro (Ückert 2001), whereas the role of other PDE families within this tissue needs to be determined. Within the bladder, unspecific blockade of different PDEs by IBMX (inhibition of PDE-1, -2, -3, -4, -5, -6, -10, and -11) could relax bladder of female Guinea Pigs whereas Zaprinast was ineffective (Gillespie 2004). Despite these inconsistent findings the role of PDE-5 in relaxation of the corpus cavernosum and the treatment of erectile dysfunction is well known and there are already very potent and selective PDE-5 inhibitors in the market. Potent and selective PDE-4 inhibitors are mainly used for the treatment of Asthma and COPD (Spina 2003).

Disclosure of the invention

One aspect of the invention is provided by a PDE mRNA expression profile demonstrating the abundance of cGMP-dependent PDE-5 and cAMP-dependent PDE-4 not only in the prostate but also in bladder tissue (Figures 1, 2). Therefore, selective inhibitors of PDE-5 or PDE-4, and in particular combinations of both, should not only reduce prostate contractility but also, as an additional benefit of a combination of both, ameliorate irritative symptoms caused by bladder outlet obstruction as it frequently occurs in urological disorders. Selective inhibitors of PDE-5 are i.e. Vardenafil, Sildenafil and Tadalafil, a selective inhibitor of PDE-4 is i.e. Roflumilast.

Urological disorders addressed by therapeutic agents of the invention comprise Benign Prostate Hyperplasia (BPH), Lower Urinary Tract Symptoms (LUTS) and in particular irritative symptoms caused by BPH-induced bladder outlet obstruction (BOO). Because not only symptomatic irritations of the bladder but also underlying BPH-induced bladder outlet obstructions are addressed by treatment with specific PDE-5 and/or PDE-4 inhibitors (and in particular combinations thereof), this treatment provides substantial advantage over methods of treatment already known in the art.

Other urological disorders which in particular and with substantial advantage can be treated by the above mentioned inhibitors, or combination of inhibitors are genitourinary disorders comprising neurogenic bladder syndrome [also referred to as overactive bladder (OAB) or interstitial cystitis (IC)], urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUT), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction.

Another aspect of the invention is the demonstration that the PDE-5 inhibitor Vardenafil has a relaxing effect on rat urethral rings with an EC_{50} value of $0.96 \mu\text{mol/l}$, and rat prostate and bladder strips with the EC_{50} value of 1.1 and $5.0 \mu\text{mol/l}$ respectively (Figure 3, Table 1).

Another aspect of the invention is the demonstration that the PDE-4 inhibitor Roflumilast and the PDE-5 inhibitor Vardenafil both show relaxing effects on rabbit bladder strips with an IC_{50} of 260 nmol/l and $1.7 \mu\text{mol/l}$ respectively (Figure 4, Table 2).

Another aspect of the invention is provided by the demonstration that the PDE-5 inhibitor Vardenafil significantly decreased the number of non-voiding contractions as a measure of irritative symptoms of BPH in the rat bladder outlet obstruction (BOO) model (Figure 5).

The invention provides PDE-5 inhibitors which are, alone or in combination with PDE-4 inhibitors, useful for the treatment of urological disorders. In particular, compounds of the invention are *Tadalafil* ((6R,12aR) -2,3,6,7,12,12a - Hexahydro - 2 - methyl - 6 - (3,4-methylene -dioxyphe-nyl) pyrazino(1',2':1,6) pyrido(3,4-b)indole-1,4-dione), *Vardenafil* (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), *Sildenafil* (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]-7-methyl-9-propyl-2,4,7,8-tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), *Udenafil* 5-[2-propyloxy-5-(1-methyl-2-pyrrolidinylethylamidosulfonyl)phenyl]-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one, *Dasantafil* 7-(3-Bromo-4-methoxybenzyl)-1-ethyl-8-[[[(1,2)-2-hydroxycyclopentyl]amino]-3-(2-hydroxyethyl)-3,7-dihydro-1-purine-2,6-dione, *Avanafil* 4-[[[(3-chloro-4-methoxyphenyl)methyl]amino]-2-[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]-N-(pyrimidin-2-ylmethyl)pyrimidine-5-carboxamide, *SLx 2101* of Surface Logix, *LAS 34179* Triazolo[1,2-]xanthine,6-methyl-4-propyl-2-[2-propoxy-5-(4-methylpiperazino)sulfonyl]phenyl-, *Roflumilast* (3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy)benzamide), *Cilomilast* (4-cyano-4-(3-

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cyclopentoxy-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid), and Piclamilast (3-cyclopentoxy-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide).

5 Still another aspect of the invention is a method of screening for PDE inhibitors, in particular for inhibitors of PDE-4- and PDE-5 for use, alone or in combination, for the preparation of medicaments for the treatment of urological disorders mentioned above.

The invention provides methods (also referred to herein as "screening assays") for identifying PDE inhibitors which can be used for the treatment of urological disorders. The methods entail the identification of candidate or test compounds or agents (e.g., peptides, 10 peptidomimetics, small molecules or other molecules) which bind to phosphodiesterases and/or have a stimulatory or inhibitory effect on the biological activity of PDE1A or its expression and then determining which of these compounds have an effect on symptoms or diseases regarding urological disorders in an in vivo assay.

15 Candidate or test compounds or agents which bind to PDE-4 or PDE-5 and/or have a stimulatory or inhibitory effect on the activity or the expression of PDE-4 or PDE-5 are identified either in assays that employ cells which express PDE-4 and/or PDE-5 (cell-based assays) or in assays with isolated PDE-4 and/or PDE-5 (cell-free assays). The various assays can employ a variety of variants of PDEs (e. g., full-length PDEs, a biologically active fragment of PDEs, or a fusion protein which includes all or a portion of PDEs). Moreover, 20 PDE-4 and/or PDE-5 can be derived from any suitable mammalian species. The assay can be a binding assay entailing direct or indirect measurement of the binding of a test compound or a known PDE-4 or PDE-5 ligand to PDE-4 or PDE-5. The assay can also be an activity assay entailing direct or indirect measurement of the activity of PDE-4 or PDE-5. The assay can also be an expression assay entailing direct or indirect measurement of the expression of 25 PDE-4 and/or PDE-5 mRNA or PDE-4 and/or PDE-5 protein. The various screening assays are combined with an in vivo assay entailing measuring the effect of the test compound on the symptoms of urological disorders.

The present invention includes biochemical, cell free assays that allow the identification of inhibitors and agonists of PDEs suitable as lead structures for pharmacological drug 30 development. Such assays involve contacting PDE-4 and/or PDE-5 with a test compound and determining the ability of the test compound to act as an antagonist (preferably) or an agonist of the enzymatic activity of PDE4 and/or PDE-5. In one embodiment, the assay includes monitoring the PDE activity of PDE-4 and/or PDE-5 by measuring the conversion

of either cAMP or cGMP to its nucleoside monophosphate after contacting PDE-4 and/or PDE-5 with a test compound.

For example, cAMP and cGMP levels can be measured by the use of the tritium containing compounds $^3\text{HcAMP}$ and $^3\text{HcGMP}$ as described in [Hansen, R. S., and Beavo, J.A., PITAS
5 USA1982,79: 2788-92]. To screen a compound pool comprised of a large number of compounds, the microtiter plate-based scintillation proximity assay (SPA) as described in [Bardelle, C. et al. (1999) Anal. Biochem. 275: 148-155] can be applied.

Alternatively, the phosphodiesterase activity of the recombinant protein can be assayed using a commercially available SPA kit (Amersham Pharmacia). The PDE enzyme
10 hydrolyzes cyclic nucleotides, e.g. cAMP and cGMP to their linear counterparts. The SPA assay utilizes the tritiated cyclic nucleotides $^3\text{HcAMP}$ or $^3\text{HcGMP}$, and is based upon the selective interaction of the tritiated non cyclic product with the SPA beads whereas the cyclic substrates are not effectively binding.

Radiolabelled product bound to the scintillation beads generates light that can be analyzed
15 in a scintillation counter.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral (e.g., intravenous, intraarterial, intradermal, subcutaneous, intramuscular, inhalative, transdermal, transmucosal, nasal and rectal administration), oral (e.g. buccal, sublingual,
20 oral mucosal and peroral administration) and local (e.g. local instillation of solutions or suspensions and local implants)

Pharmaceutical compositions suitable for injections and infusions include sterile aqueous solutions (if the active ingredient is sufficiently soluble in water), suspensions, emulsions and sterile powders for the extemporaneous preparation of sterile injectable solutions or
25 dispersions. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyethyleneglycol, and suitable mixtures thereof. Pharmaceutically acceptable ingredients may be added like buffers, preservatives, antioxidants, isotonicizing agents or surfactants. Depot injections are based on known formulations principles like oily solutions or
30 suspensions or particles of biodegradable polymers.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant from a nebulizer or a dry powder inhaler.

5 Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays, sublingual or buccal preparations or suppositories. For transdermal administration,
10 the active compounds are formulated into ointments, patches, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

15

Oral compositions generally include an inert diluent or bulking agent and functional excipients. They can be enclosed in capsules or compressed into tablets. Other suitable dosage forms are effervescent tablets, chewable tablets, orodispersible tablets, softgelatine capsules, liquid filled hardgelatine capsules, powders in sachets and oral liquids.

20

Suitable functional excipients for the preparation of oral dosage forms are well known in the art and include, for example, binders such as polyvinylpyrrolidone or hydroxypropylmethylcellulose, disintegrants such as crospovidone or croscarmellose sodium, glidants like colloidal silicium dioxide, lubricants such as magnesium stearate, macrogols or stearic acid, sweetening agents such as aspartame, sucrose or saccharin sodium
25 and flavouring agents such as peppermint or orange flavouring.

25

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as controlled release tablets or coated pellets filled in capsules or parenteral controlled release formulations, including implants and microencapsulated delivery systems. Biodegradable or biocompatible polymers
30 can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid or polyglycolic-polylactic-copolymers.

30

In another embodiment the invention provides combinations of PDE-4 and PDE-5 inhibitors and their use for the preparation of pharmaceutical compositions for the treatment of urological disorders, whereby these combinations comprise either i) pharmaceutical compositions comprising a compound having inhibitory action on both PDE-4 and PDE-5 activity, or ii) pharmaceutical compositions comprising at least one PDE-4 inhibitor and at least one PDE-5 inhibitor as a fixed combination in one application unit, or iii) a kit of parts containing at least two sets of pharmaceutical compositions, each set consisting of at least one pharmaceutical preparation comprising a PDE-5 inhibitor in units of at least one dose and at least one pharmaceutical preparation comprising a PDE-4 inhibitor in units of at least one dose, whereby each application unit of said pharmaceutical compositions is administered in combination, sequentially, as single dose or in multiple doses.

The present invention provides further:

A method of screening for PDE 5 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a test compound (which may or may not have PDE-4 inhibitory activity) with a PDE5 polypeptide, ii) determining the activity of the PDE5 polypeptide at a certain concentration of the test compound or in the absence of said test compound, iii) determining the activity of said PDE5 polypeptide at a different concentration of said test compound, iv) selecting at least one compound with inhibitory effect on the PDE-5 polypeptide.

A method of screening for PDE 4 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a test compound (which may or may not have PDE-5 inhibitory activity) with a PDE4 polypeptide, ii) determining the activity of the PDE4 polypeptide at a certain concentration of the test compound or in the absence of said test compound, iii) determining the activity of said PDE4 polypeptide at a different concentration of said test compound, iv) selecting at least one compound with inhibitory effect on the PDE-4 polypeptide.

A method of screening for combinations of PDE 5 inhibitors and PDE 4 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower

Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a first test compound with a PDE5 polypeptide, ii) determining the activity of the PDE5 polypeptide at a certain concentration of the first test compound or in the absence of said first test compound, iii) determining the activity of the PDE5 polypeptide at a different concentration of said first test compound, iv) selecting at least one first compound with inhibitory effect on the PDE-5 polypeptide, v) contacting a second test compound with a PDE4 polypeptide, vi) determining the activity of the PDE4 polypeptide at a certain concentration of the second test compound or in the absence of said second test compound, vii) determining the activity of the PDE4 polypeptide at a different concentration of said second test compound, vii) selecting at least one second compound with inhibitory effect on the PDE-4 polypeptide, vii) combining at least one first compound with PDE5 inhibitory activity with at least one second compound having PDE4 inhibitory activity.

Methods of screening which involve contacting the test compound in or at the surface of a cell, wherein the cell is in vitro.

Methods of screening which involve contacting the test compound with the PDE-4 or PDE-5 polypeptide in a cell free system.

Methods of screening may involve a test compound which is coupled to a detectable label.

In particular, the present invention provides:

A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO), Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UII, SUI, OUI), pelvic pain, benign and malignant disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy and obstructive uropathies in a mammal, comprising a therapeutic agent which regulates the activity of a PDE5 polypeptide.

A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction

(BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which regulates the activity of a PDE4 polypeptide.

5 A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which is a combination of the above mentioned selective therapeutic agents.

10 A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which regulates the activity of a PDE5 polypeptide and a PDE4 polypeptide.

15 A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a PDE-4 inhibitor selected from the group of PDE-4 Inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy) -N-(3,5-dichloropyridin-4-yl)-4-(difluoro-methoxy)benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxymethoxy-phenyl)-cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxymethoxy-N-(3,5-dichloropyridin-4-yl)-4-methoxybenzamide).

20 A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like
25 renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy and obstructive uropathies in a mammal comprising a PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of *Tadalafil* ((6R,12aR) -2,3,6,7,12,12a - Hexahydro - 2 - methyl - 6 - (3,4-methylene - dioxyphe-
30 nyl) pyrazino(1',2':1,6) pyrido(3,4-b)indole-1,4-dione), *Vardenafil* (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl)-1-sulfonylphenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), *Sildenafil* (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonylphenyl]-7-methyl-9-propyl-2,4,7,8-tetrazabicyclo [4.3.0]nona-3,8,10-trien-5-one), *Udenafil* 5-[2-propyloxy-5-(1-methyl-2-pyrrolidinyl-ethyl-amidosulfonyl)phenyl]-methyl-3-propyl-1,6-

5 dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one, *Dasantafil* 7-(3-Bromo-4-methoxybenzyl)-1-ethyl-8-[[[(1,2)-2-hydroxycyclopentyl]amino]-3-(2-hydroxyethyl)-3,7-dihydro-1-purine-2,6-dione, *Avanafil* 4-[[[(3-chloro-4-methoxy phenyl)methyl]amino]-2-[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]-N-(pyrimidin-2-yl methyl)pyrimidine-5-carboxamide, *SLx 2101* of Surface Logix, *LAS 34179* Triazolo[1,2-]xanthine, 6-methyl-4-propyl-2-[2-propoxy-5-(4-methylpiperazino)-sulfonyl]phenyl or salts, hydrates or hydrates of salts thereof.

10 A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a combination of at least one PDE-4 inhibitor selected from the group of PDE-4 inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxo-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxo-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide) and at least one PDE-5 inhibitor selected from the group of PDE-5 inhibitors consisting of Vardenafil (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl)-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]- 7- methyl - 9- propyl - 2,4,7,8- tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), and Tadalafil ((6R,12aR) -2,3,6,7, 12, 12a- Hexahydro - 2 - methyl - 6 - (3,4-methylene-dioxyphenyl).

20 Use of a PDE5 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, 25 SUI, OUI), pelvic pain, benign and malignant disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy and obstructive uropathies in a mammal.

30 Use of a PDE4 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

Use of a combination of at least one PDE4 inhibitor and at least one PDE5 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction in a mammal.

Use of an agent which is a inhibitor of a PDE4 polypeptide and a PDE5 polypeptide for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

Use of PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of *Tadalafil* ((6R,12aR) -2,3,6,7,12,12a - Hexahydro - 2 - methyl - 6 - (3,4-methylene -dioxyphe-nyl) pyrazino(1',2':1,6) pyrido(3,4-b)indole-1,4-dione), *Vardenafil* (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), *Sildenafil* (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]-7-methyl-9-propyl-2,4,7,8-tetrazabicyclo [4.3.0]nona-3,8,10-trien-5-one), *Udenafil* 5-[2-propyloxy-5-(1-methyl-2-pyrrolidinyl-ethyl-amidosulfonyl)phenyl]-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one, *Dasantafil* 7-(3-Bromo-4-methoxybenzyl)-1-ethyl-8-[[[(1,2)-2-hydroxycyclopentyl]amino]-3-(2-hydroxyethyl)-3,7-dihydro-1-purine-2,6-dione, *Avanafil* 4-[[[(3-chloro-4-methoxy phenyl)methyl]amino]-2-[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]-N-(pyrimidin-2-yl methyl)pyrimidine-5-carboxamide, *SLx 2101* of Surface Logix, *LAS 34179* Triazolo[1,2-]xanthine, 6-methyl-4-propyl-2-[2-propoxy-5-(4-methylpiperazino)-sulfonyl]phenyl or salts, hydrates or hydrates of salts thereof, for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUT), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male,

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renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy and obstructive uropathies in a mammal.

5 Use of PDE-4 inhibitor selected from the group of PDE-4 Inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxo-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxo-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide) for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH),
10 Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

Use of a combination of at least one PDE-4 inhibitor selected from the group of PDE-4 inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxo-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid) and Piclamilast (3-cyclopentoxo-N-(3,5-dichloropyridin-4-yl)-4-methoxy-benzamide) and at least one PDE-5 inhibitor selected from the group of PDE-5 inhibitors consisting of Vardenafil (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl)-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4) triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl] - 7-methyl-9-propyl-2,4,7,8-tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), and Tadalafil ((6R,12aR) -2,3,6,7,12,12a-Hexahydro -2-methyl-6-(3,4-methylene-dioxyphenyl) for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction in a mammal.
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A Method for the preparation of a pharmaceutical composition useful for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS),

genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, LUI, SUI, OUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction in a mammal comprising the steps of i) identifying a inhibitor of PDE5 according to the method of screening described above ii) identifying a inhibitor of PDE4 according to the method of screening described above) determining whether said inhibitors ameliorate the symptoms of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal; and iii) combining at least one of said inhibitors with an acceptable pharmaceutical carrier.

A method for the preparation of a pharmaceutical composition wherein the inhibitor of PDE5 is a PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of Vardenafil (2-(2-Ethoxy-5-(4-ethylpiperazin-1-yl-1-sulfonyl)phenyl)-5-methyl-7-propyl-3H-imidazo (5,1-f) (1,2,4)triazin-4-one), Sildenafil (3-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonyl-phenyl]- 7- methy l- 9- propy l-2,4,7,8- tetrazabicyclo [4.3.0]nona -3,8,10-trien-5-one), Tadalafil ((6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl), Udenafil 5-[2-propyloxy-5-(1-methyl-2-pyrrolidinyl-ethyl-amidosulfonyl)phenyl]-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one, Dasantaflil 7-(3-Bromo-4-methoxybenzyl)-1-ethyl-8-[[[(1,2)-2-hydroxycyclopentyl]amino]-3-(2-hydroxyethyl)-3,7-dihydro-1-purine-2,6-dione, Avanafil 4-[[[(3-chloro-4-methoxyphenyl)methyl]amino]-2-[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]-N-(pyrimidin-2-ylmethyl)pyrimidine-5-carboxamide, SLx 2101 of Surface Logix and LAS 34179Triazolo[1,2-xanthine,6-methyl-4-propyl-2-[2-propoxy-5-(4-methylpiperazino)-sulfonyl]phenyl.

A method for the preparation of a pharmaceutical composition wherein the inhibitor of PDE4 is a PDE-4 inhibitor selected from the group of PDE-4 Inhibitors consisting of Roflumilast (3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy)benzamide), Cilomilast (4-cyano-4-(3-cyclopentoxy-4-methoxy-phenyl)-cyclohexane-1-carboxylic acid) and Pielamilast (3-cyclopentoxy-N-(3,5-dichloropyridin-4-yl)-4-methoxybenzamide).

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Use of a pharmaceutical composition as mentioned above for the regulation of PDE activity in a mammal having a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction.

A kit of parts for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal including humans containing a combination of at least one pharmaceutical composition selected from the group of pharmaceutical compositions consisting of Vardenafil, Sildenafil and Tadalafil and at least one pharmaceutical composition selected from the group of pharmaceutical compositions consisting of Roflumilast, Cilomilast and Piclamilast.

A Method for the preparation of a kit of parts useful for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising the steps of i) selecting at least one pharmaceutical composition from the group of pharmaceutical compositions consisting of Vardenafil, Sildenafil and Tadalafil ii) selecting at least one pharmaceutical composition from the group of pharmaceutical compositions consisting of Roflumilast, Cilomilast and Piclamilast, iii) combining at least two of said pharmaceutical compositions thereby creating said kit of parts.

A kit of parts for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal containing a combination of at least one therapeutic agent regulating the activity of a PDE-5 polypeptide and Tadalafil and at least one therapeutic agent regulating the activity of a PDE-4 polypeptide.

A Method for the preparation of a kit of parts useful for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising

the steps of i) selecting at least one pharmaceutical composition comprising a therapeutic agent regulating the activity of a PDE-5 polypeptide, ii) selecting at least one pharmaceutical composition comprising a therapeutic agent regulating the activity of a PDE-4 polypeptide, iii) combining at least two of said pharmaceutical compositions thereby
5 creating said kit of parts.

A preferred embodiment of the invention is a pharmaceutical composition containing Vardenafil, or a salt, a hydrate or a hydrate of a salt thereof, for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO), Lower Urinary Tract Symptoms (LUTS), genitourinary disorders
10 comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases,
15 glomerulopathies, nephritis, toxic nephropathy and obstructive uropathies in a mammal.

It has surprisingly be found that especially Vardenafil, or a salt, a hydrate or a hydrate of a salt thereof, exhibit higher activities and shows better results in the treatment of neurogenic bladder (also referred to as overactive bladder or interstitial cystitis) compared with other PDE-5 inhibitors.

Description of figures

Figure 1: Relative mRNA Expression of PDE-5 in Kindney (K), Bladder (B), Prostate (P), Urethra (U) and Corpus Cavemosum (C) of Sprague Dawley Rats. Data are mean + SEM, n=10.

5 **Figure 2:** Relative mRNA Expression of PDE-4a, -4b, -4c, -4d and PDE-5 in bladder and prostate of Sprague Dawley Rats. Data are mean + SEM, n=10.

Figure 3: Effects of Vardenafil on the contraction of isolated rat urethral rings (black triangles), and bladder- (black diamonds) and prostate strips (grey squares). The bladder strips were pre-contracted using K^+ (50 mmol/l) Krebs-Henseleit solution. Prostate and urethral tissues were pre-contracted using 10 μ mol/l Phenylephrine. The relaxation was expressed as percentage of the pre-contraction. Each point represents a mean value $\pm$ SEM. n = 9.

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Figure 4: Effects of Roflumilast (black diamonds) and Vardenafil (grey squares) on the contraction of isolated rabbit bladder strips. The bladder strips were pre-contracted using K^+ (50 mmol/l) Krebs-Henseleit solution. The relaxation was expressed as percentage of the pre-contraction. Each point represents a mean value $\pm$ SEM. n = 9.

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Figure 5: Number of non-voiding contractions in % after bolus i.v. treatment with vehicle (V) and Vardenafil HCl (1, 3 and 10 mg/kg). Data are mean + SEM, * = significant with $p < 0.05$ (paired student's t-test).

20 **Figure 6:** Micturition interval in % after bolus i.v. treatment of vehicle (V) and Vardenafil HCl (1,3 mg/kg) compared to the basal micturition interval (C). Data are mean + SEM, * = significant with $p < 0.05$ (paired student's t-test).

Example 1

Tissue sampling and RNA preparation: Male Sprague Dawley rats with a body weight between 200-250 g were used for tissue collecting. The rats were briefly anaesthetized with a mixture of 5% Isoflurane (Baxter S.A.) in a carrier with 70% N_2O and 30% O_2 , and than euthanized by decapitation. The abdomen was opened by a midline incision and the kidneys and lower urinary tract tissue as renal medulla, urether, bladder prostate and urethra were exposed and quickly removed. The tissues were frozen in liquid N_2 and stored until RNA

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preparation. Total RNA was isolated using RNeasy mini columns (Qiagen Inc.) and further purified by DNase digestion.

PDE mRNA quantification: The mRNA expression of the different PDE isogenes in rat lower urinary rat tissues was measured by real time quantitative PCR (TaqMan-PCR, Heid 5 1996). Therefore 1 µg of total RNA were transcribed into cDNA with Superscript II RT cDNA synthesis kit (Gibco, Inc) according to the manual of the supplier. The mRNA for the PDEs were measured by real-time quantitative RT-PCR on an ABI Prism 7700 sequence detection instrument (Applied Biosystems, Inc.). Specific Sequences for forward and reverse primers as for the fluorogenic probe of each PDE isogene mRNA were designed by Primer 10 Express 1.5 Software (Applied Biosystems, Inc.). During PCR amplification, 5' nucleolytic activity of Taq polymerase cleaves the probe separating the 5' reporter fluorescent dye from the 3' quencher dye. The threshold cycle, Ct, which correlates inversely with the target mRNA level, was measured as the cycle number at which the reporter fluorescent emission increases 10 standard deviations above background level. As housekeeping gene, beta-actin 15 was quantified as described above, using as forward primer 5'-accttcaacaccccagcca-3', reverse primer 5'-cagtggtagcagcaggagca-3' and fluorescent probe 5'-6AFM-acgtagccatccaggctgtgtgtcc-TAMARA-3'. The PDE mRNA levels were corrected for beta-actin mRNA levels and calculated as relative expression using comparative Ct-method.

Expression of PDE-5 and PDE-4A, -4B, -4C, -4D mRNA in the lower urinary tract: Since 20 there is only incomplete data on the expression profile of PDE-5 in lower urinary tract tissue, PDE mRNA was quantified in male Sprague Dawley rats via TaqMan RT-PCR. The most prominent expression of PDE-5 was found in the bladder (Figure 1). Lower expression levels were found in the urethra, the corpus cavernosum and the prostate (Figure 1). These result showed that there is a substantial PDE-5 mRNA expression in lower urinary tract 25 tissues especially in the bladder.

Moreover PDE-4 mRNA expression of all four PDE-4 isogenes (PDE-4A, -4B, -4C and -4D) was determined by TaqMan RT-PCR in the bladder and the prostate (Figure 2). We found very low expression of PDE-4c, which was on the borderline of detectability, however PDE-4A, -4B and -4D mRNAs were substantially expressed within both tissues. In the bladder 30 PDE-4D was the most abundant PDE-4 isogene mRNA whereas in the prostate PDE-4a and -4D are almost equally distributed and 2.5-fold higher expressed than PDE-5 mRNA (Figure 2).

The expression profile demonstrates that PDE-5 and PDE-4D mRNAs are abundant in the bladder but also in prostate tissue. Therefore, inhibitors of PDE-5 or PDE-4, but in particular combinations of both, PDE-5 and PDE-4 inhibitors, such as Vardenafil in combination with Roflumolast should not only reduce bladder but also prostate contractility, thus providing advantage over methods of treatment for urological disorders already existing in the state of the art, said disorders comprising i. e. Benign Prostate Hyperplasia (BPH) and in particular irritative symptoms caused by BPH-induced bladder outlet obstruction (BOO) including, but not limited to, Lower Urinary Tract Symptoms (LUTS).

Example 2

Tissue preparation: Male Wistar rats (200-300 g) were euthanized using carbon dioxide. The tissues were removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl₂ 2.0 MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, glucose 11.5. Four equally sized longitudinal strips of approximately 2 mm x 10 mm were cut from the bladder body. Prostate strips were obtained by cutting transversally through the lobes of the prostate gland parallel to the urethra. One ring per animal was dissected from the proximal part of the urethra.

White New Zealand rabbits were anesthetized using thiopental. The urinary bladder was removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl₂ 2.0 MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, and glucose 11.5. Equally sized longitudinal strips of approximately 2 mm x 10 mm were cut from the bladder body.

Recording of mechanical activity: The preparations were transferred to 20 ml organ baths containing Krebs-Henseleit solution equilibrated with 95% O₂, 5% CO₂ at 37°C. The strips were mounted between two hooks by means of two clips. For recording of isometric tension one of the hooks was connected to a force transducer which was in turn linked to an amplifier and chart recorder. The other hook was attached to a movable unit, permitting precise adjustment of preload tension. All tissues were then given a 60 min equilibration period during which they were washed and the resting tension was adjusted to 1 g every 20 min.

After the equilibration period, each experiment was started by exposing the preparation to K^+ (50 mmol/l) Krebs-Henseleit solution. The procedure was repeated 3 times and the tissues were washed at least three times between each contraction.

The bladder strips were then pre-contracted using K^+ (50 mmol/l) Krebs-Henseleit solution.

When the contraction was stabilized, an accumulative dose response curve of the compound tested was constructed. The stabilized contraction induced by K^+ (50 mmol/l) Krebs-Henseleit solution was defined as 100% tension. The relaxation was expressed as percentage tension.

Prostatic strips and urethral rings were pre-contracted using 10^{-6} mol/l phenylephrine. The effects of the compounds on the prostate tissue were tested in a non-cumulative manner with washing steps between each concentration.

Organ bath assay: effects of Vardenafil on isolated rat urogenital organs: The effects of the PDE5 inhibitor Vardenafil on the relaxation of smooth muscles were tested in the organ bath system. The compound was applied in the concentration range from 10^{-8} mol/l to 10^{-5} mol/l (Figure 3, Table 1). Vardenafil relaxed the urethral rings with an EC_{50} value of 0.96 μ mol/l, and the prostate and bladder strips with the EC_{50} value of 1.1 and 5.0 μ mol/l respectively.

Concentration (μ mol/l)	Bladder (% contraction)	Urethra (% contraction)	Prostate (% contraction)
0.001	94.3 $\pm$ 0.9	88.4 $\pm$ 2.4	-
0.01	91.2 $\pm$ 1.3	88.5 $\pm$ 2.1	-
0.1	89.9 $\pm$ 1.8	77.1 $\pm$ 3.9	99.2 $\pm$ 3.0
1	77.8 $\pm$ 2.1	44.9 $\pm$ 1.5	76.0 $\pm$ 2.3
10	25.3 $\pm$ 3.2	3.5 $\pm$ 1.7	25.3 $\pm$ 3.2

Table 1: Effects of vardenafil on the contraction of isolated rat urogenital tissues. The relaxation is expressed as percentage of the pre-contraction. Each point represents a mean value $\pm$ SEM. $n = 9$.

Example 3

Tissue preparation: Male Wistar rats (200-300 g) were euthanized using carbon dioxide. The tissues were removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, $CaCl_2$ 2.0 $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$

25, glucose 11.5. Four equally sized longitudinal strips of approximately 2 mm x 10 mm were cut from the bladder body. Prostate strips were obtained by cutting transversally through the lobes of the prostate gland parallel to the urethra. One ring per animal was dissected from the proximal part of the urethra.

5 White New Zealand rabbits were anesthetized using thiopental. The urinary bladder was removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl₂ 2.0 MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, and glucose 11.5. Equally sized longitudinal strips of approximately 2 mm x 10 mm were cut from the bladder body.

10 Recording of mechanical activity: The preparations were transferred to 20 ml organ baths containing Krebs-Henseleit solution equilibrated with 95% O₂, 5% CO₂ at 37°C. The strips were mounted between two hooks by means of two clips. For recording of isometric tension one of the hooks was connected to a force transducer which was in turn linked to an amplifier and chart recorder. The other hook was attached to a movable unit, permitting
15 precise adjustment of preload tension. All tissues were then given a 60 min equilibration period during which they were washed and the resting tension was adjusted to 1 g every 20 min.

After the equilibration period, each experiment was started by exposing the preparation to K⁺ (50 mmol/l) Krebs-Henseleit solution. The procedure was repeated 3 times and the
20 tissues were washed at least three times between each contraction.

The bladder strips were then pre-contracted using K⁺ (50 mmol/l) Krebs-Henseleit solution. When the contraction was stabilized, an accumulative dose response curve of the compound tested was constructed. The stabilized contraction induced by K⁺ (50 mmol/l) Krebs-Henseleit solution was defined as 100% tension. The relaxation was expressed as percentage
25 tension.

Prostatic strips and urethral rings were pre-contracted using 10⁻⁶ mol/l phenylephrine. The effects of the compounds on the prostate tissue were tested in a non-cumulative manner with washing steps between each concentration.

Effects of PDE5 and PDE4 inhibitors on isolated rabbit bladder strips: The effects of the
30 PDE5 inhibitor vardenafil and the PDE4 inhibitor Roflumilast on the relaxation the bladder smooth muscle was tested in the organ bath using rabbit bladder strips. Both compounds

were tested in the concentration from 10^{-9} mol/l to 10^{-5} mol/l (Fig. 4, Tab. 2). Roflumilast and vardenafil both relaxed the bladder strips with an IC₅₀ of 260 nmol/l and 1.7 μ mol/l respectively. (Table 2)

Concentration (μ mol/l)	Roflumilast (% contraction)	Vardenafil (% contraction)
0.001	100.8 $\pm$ 0.6	100.9 $\pm$ 1.0
0.01	90.1 $\pm$ 1.7	99.9 $\pm$ 0.9
0.1	75.1 $\pm$ 2.1	95.7 $\pm$ 1.7
1	55.8 $\pm$ 2.5	76.3 $\pm$ 3.7
10	40.9 $\pm$ 3.3	9.6 $\pm$ 12.6

5 Table 2: Effects of Roflumilast and Vardenafil on the contraction of isolated rabbit bladder strips. The relaxation is expressed as percentage of the pre-contraction. Each point represents a mean value $\pm$ SEM. n = 9.

Example 4

10 All animal experiments were performed due to the "German Law for the Protection of Laboratory animals" and were conducted due to the approved guidelines of the permission "Tierversuchsvorhaben No 401/A01 M010/M011 vom 09.07.2004". Experiments were performed with female Sprague Dawley Rats with a body weight between 200-250g.

15 Bladder Outlet Obstruction: For the bladder outlet obstruction, rats were anesthetized with a mixture of 1.5-2% isoflurane in a carrier of 66% N₂O 33% O₂. The abdomen was shaved, opened by a lower midline incision, bladder and urethra were identified and the urethra-vesical junction was exposed. A 1.0 mm metal rod was placed along the proximal urethra and a 6-0 nylon ligature was tied tightly around the urethra and the rod. The rod was consecutively removed and the abdomen was closed by a silk ligature and cleaned up by 70% ethanol. There was a postoperative anti-pain treatment with 10 mg/kg Rimadyl®
20 (Pfizer). Rats were kept then for 2 weeks and feed with tap water and standard rat chow. 24 hours prior to the cystometry rats were anaesthetized with isoflurane as described above. The laparotomy was performed as described above, the bladder was exposed and a polyethylene catheter (PE50) was implanted into the bladder dome. The catheter was tunneled subcutaneously using a cannula to reach the back neck of the animal. Additionally,
25 a catheter for intravenous administration (PE10) was placed into the jugular vein and

tunneled subcutaneously to the back neck of the animal. Both catheters were fixed by a suture and a tape.

Conscious cystometry: For cystometry the animals were shortly anaesthetized by isoflurane as described above, placed in a Ballman's cage and fixed. Then animals were recovered at least for 1 h before the experiment started. The bladder catheter was then connected to the t-shaped tube to connect a pressure transducer for measurement of intra-bladder pressure (MLT0698, ADInstruments) and an infusion pump (Perfusor Compact®, Braun Melsungen) for continuous infusion of saline solution at a flow rate of 10 ml/h. The BOO animals showed an increase in bladder capacity (due to the bladder enlargement) and non voiding contractions (mimicking the irritative symptoms of BPH), when compared with a control animal. The efficacy of treatment was quantified via calculation of the non voiding contractions per micturition interval before and after treatment. For positive control the alpha receptor antagonist tamsulosin (10 µg/kg) was used. Values were given in % reduction of non voiding contractions.

Statistical analysis of results: Data are expressed as means ± standard error of the means (SEM), and *n* indicating the number of experiments. The significance of differences between means was determined by paired and unpaired Student's t-test. Probability levels less than 0.05 were considered significant.

Effect of vardenafil on non voiding contractions in BOO rats: For the BOO model a partial ligature of the urethra was performed in rat under anesthesia. The bladder outlet obstruction (BOO) resulting from this procedure caused a significant increase of bladder weight (data not shown) indicating a pronounced bladder hypertrophy. It also caused non voiding contractions (NVC) of the bladder which were detected via cystometry in conscious animals. These NVC were a measure of irritative symptoms in BPH and were significantly reduced with an MED of 3mg/kg Vardenafil i.v..

Example 5

All animal experiments were performed due to the "German Law for the Protection of Laboratory animals" and were conducted due to the approved guidelines of the permission "Tierversuchsvorhaben No 401/A01 M010/M011 vom 09.07.2004". Experiments were performed with female Sprague Dawley Rats with a body weight between 200-250g.

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Anaesthetized cystometry: For cystometry female SD rats were anaesthetized with urethane (1.2g/kg, ip). After laparotomy, the bladder was exposed and both ureters were ligated and cut. A polyethylene cannula (PE50) was implanted into the bladder dome, and the abdomen was closed. The bladder catheter was connected to the t-shaped tube to connect a infusion pump (Perfusor® compact; Braun Melsungen) for continuous infusion of saline solution and to connect a pressure transducer (Combitrans; Braun Melsungen) for measurement of intrabladder pressure. The intrabladder pressure signals were registered with the Powerlab System (MLT0698, ADInstrument). Cystometry was performed after 1 hr equilibration period from the surgical procedure. For i.v. drug treatment, the left femoral vein was cannulated with a polyethylene catheter. The effect of treatments was calculated on the micturition interval (corresponding to bladder capacity).

Induction of an overactive bladder was performed with 0.2% acetic acid solution (diluted with saline) infusion into the bladder instead of saline solution or with i.p. injection of 150mg/kg of cyclophosphamide 18 hr before cystometry.

Statistical analysis of results: Data are expressed as means $\pm$ standard error of the means (SEM), and n indicating the number of experiments. The significance of differences between means was determined by paired and unpaired Student's t-test. Probability levels less than 0.05 were considered significant.

Effect of vardenafil on micturition interval in CYP-treated rats: The micturition interval was significantly increased with an MED of 3mg/kg Vardenafil i.v..

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Claims

1. A method of screening for PDE 5 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a test compound with a PDE5 polypeptide, ii) determining the activity of the PDE5 polypeptide at a certain concentration of the test compound or in the absence of said test compound, iii) determining the activity of said PDE5 polypeptide at a different concentration of said test compound, iv) selecting at least one compound with inhibitory effect on the PDE-5 polypeptide.
2. A method of screening for PDE 4 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a test compound with a PDE4 polypeptide, ii) determining the activity of the PDE4 polypeptide at a certain concentration of the test compound or in the absence of said test compound, iii) determining the activity of said PDE4 polypeptide at a different concentration of said test compound, iv) selecting at least one compound with inhibitory effect on the PDE-4 polypeptide.
3. A method of screening for combinations of PDE 5 inhibitors and PDE 4 inhibitors useful as therapeutic agents in the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) comprising the steps of i) contacting a first test compound with a PDE5 polypeptide, ii) determining the activity of the PDE5 polypeptide at a certain concentration of the first test compound or in the absence of said first test compound, iii) determining the activity of the PDE5 polypeptide at a different concentration of said first test compound, iv) selecting at least one first compound with inhibitory effect on the PDE-5 polypeptide, v) contacting a second test compound with a PDE4 polypeptide, vi) determining the activity of the PDE4 polypeptide at a certain concentration of the second test compound or in the absence of said second test compound, vii) determining the activity of the PDE4 polypeptide at a different concentration of said second test compound, vii) selecting at least one second compound with inhibitory

effect on the PDE-4 polypeptide, vii) combining at least one compound with PDE5 inhibitory activity with at least one compound having PDE4 inhibitory activity.

4. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction in a mammal comprising a therapeutic agent which regulates the activity of a PDE5 polypeptide.
5. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which regulates the activity of a PDE4 polypeptide.
6. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal comprising a therapeutic agent which is a combination of the therapeutic agents of claims 4 and 5.
7. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction in a mammal comprising a PDE-5 inhibitor selected from the group of PDE-5 Inhibitors

consisting of Vardenafil, Sildenafil, Tadalafil, Udenafil, Dasantafil, Avanafil, SLx2101 and LAS34179.

8. Use of a PDE5 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UUI, SUI, OUI), pelvic pain, benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction in a mammal.
9. Use of a PDE4 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.
10. Use of a combination of at least one PDE4 inhibitor and at least one PDE5 inhibitor for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.
11. Use of an agent which is a inhibitor of a PDE4 polypeptide and a PDE5 polypeptide for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.
12. Use of PDE-5 inhibitor selected from the group of PDE-5 Inhibitors consisting of Vardenafil, Sildenafil, Tadalafil, Udenafil, Dasantafil, Avanafil, SLx2101 and LAS34179 for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract

Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UII, SUI), pelvic pain, benign and malignant disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction in a mammal.

13. Use of a combination of at least one PDE-4 inhibitor selected from the group of PDE-4 inhibitors consisting of Roflumilast, Cilomilast and Piclamilast and at least one PDE-5 inhibitor selected from the group of PDE-5 inhibitors consisting of Vardenafil, Sildenafil and Tadalafil for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO) and Lower Urinary Tract Symptoms (LUTS) in a mammal.

14. A pharmaceutical composition containing at least one compound selected from the group Vardenafil, Sildenafil, Tadalafil, Udenafil, Dasantafil, Avanafil, SLx2101 and LAS34179 or a salt, a hydrate or a hydrate of a salt thereof, for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO), Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (OAB) and (IC), urinary incontinence (UI) like mixed-, urge-, stress-, or overflow incontinence (MUI, UII, SUI), pelvic pain, benign and malignant disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy and obstructive uropathies in a mammal.

15. A pharmaceutical composition containing at least one compound selected from the group Vardenafil, Sildenafil, Tadalafil, Udenafil, Dasantafil, Avanafil, SLx2101 and LAS34179 or a salt, a hydrate or a hydrate of a salt thereof, for the treatment of neurogenic bladder, overactive bladder and interstitial cystitis.

16. A pharmaceutical composition containing Vardenafil or a salt, a hydrate or a hydrate of a salt thereof, for the treatment of neurogenic bladder, overactive bladder and interstitial cystitis.
- 5 17. Use of at least one compound selected from the group Vardenafil, Sildenafil, Tadalafil, Udenafil, Dasantafil, Avanafil, SLx2101 and LAS34179 or a salt, a hydrate or a hydrate of a salt thereof, for the preparation of a pharmaceutical composition for the treatment of neurogenic bladder, overactive bladder and interstitial cystitis.
- 10 18. Use of Vardenafil or a salt, a hydrate or a hydrate of a salt thereof, for the preparation of a pharmaceutical composition for the treatment of neurogenic bladder, overactive bladder and interstitial cystitis.

Fig. 1

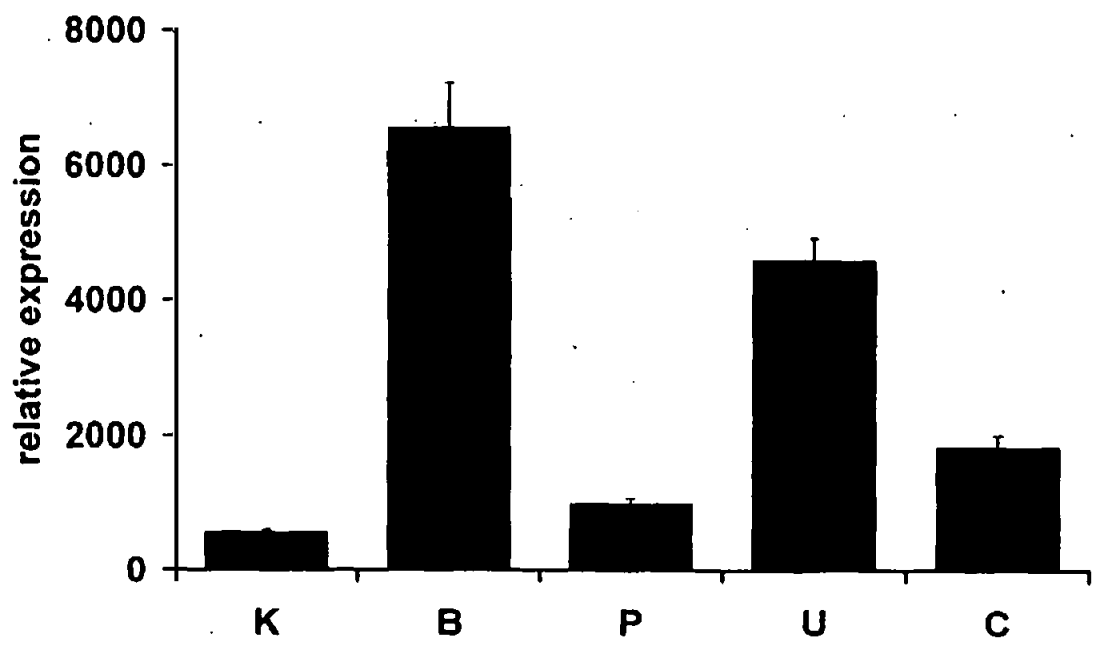


Fig. 2

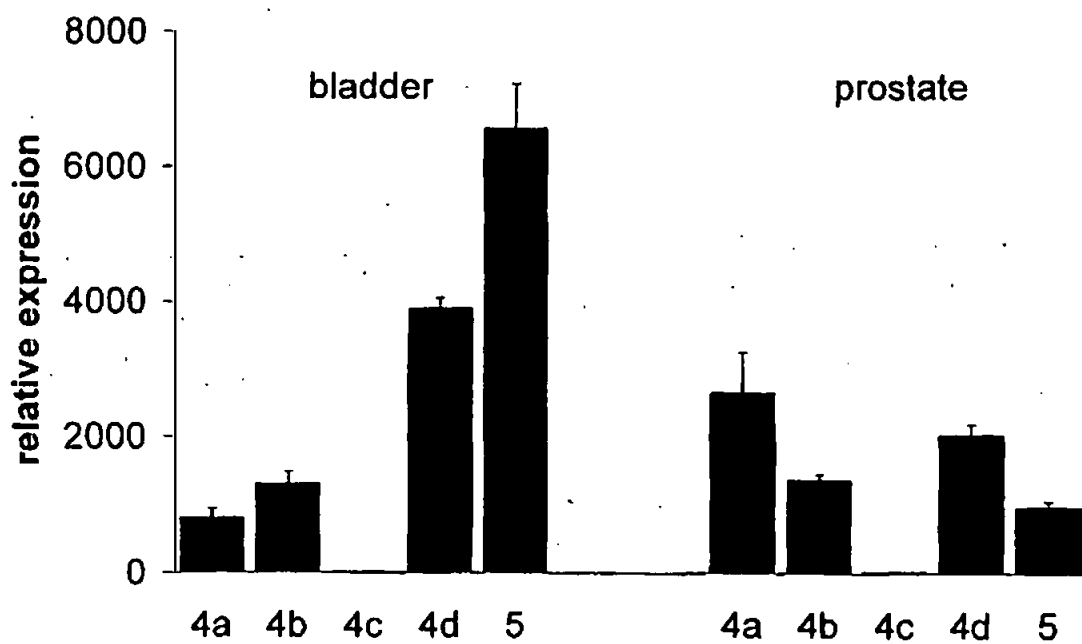
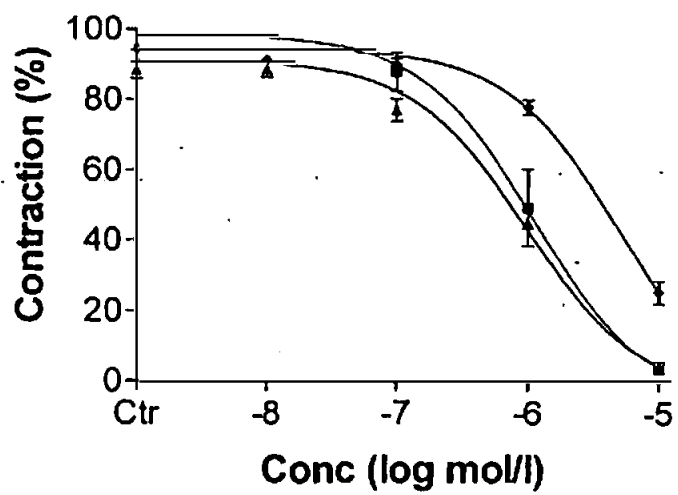
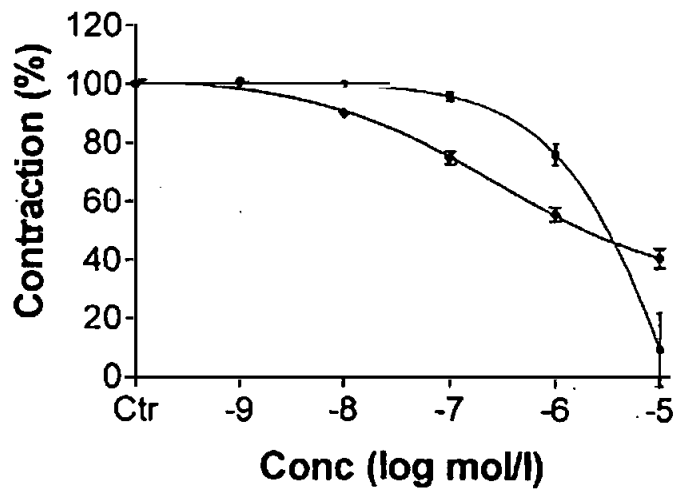


Fig. 3



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Fig.4



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Fig.5

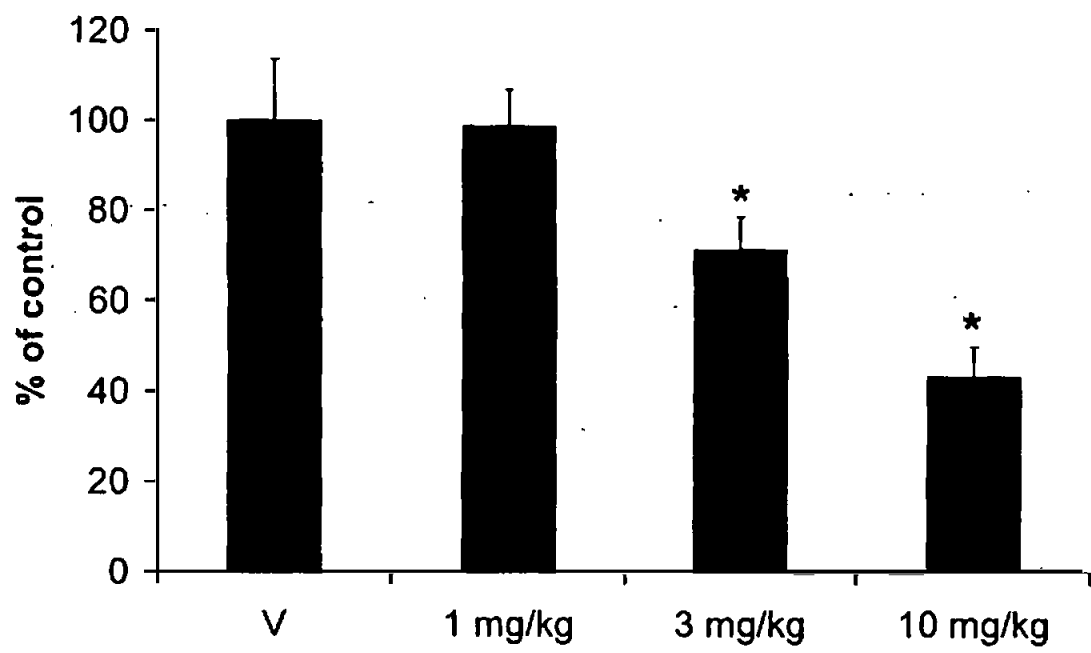
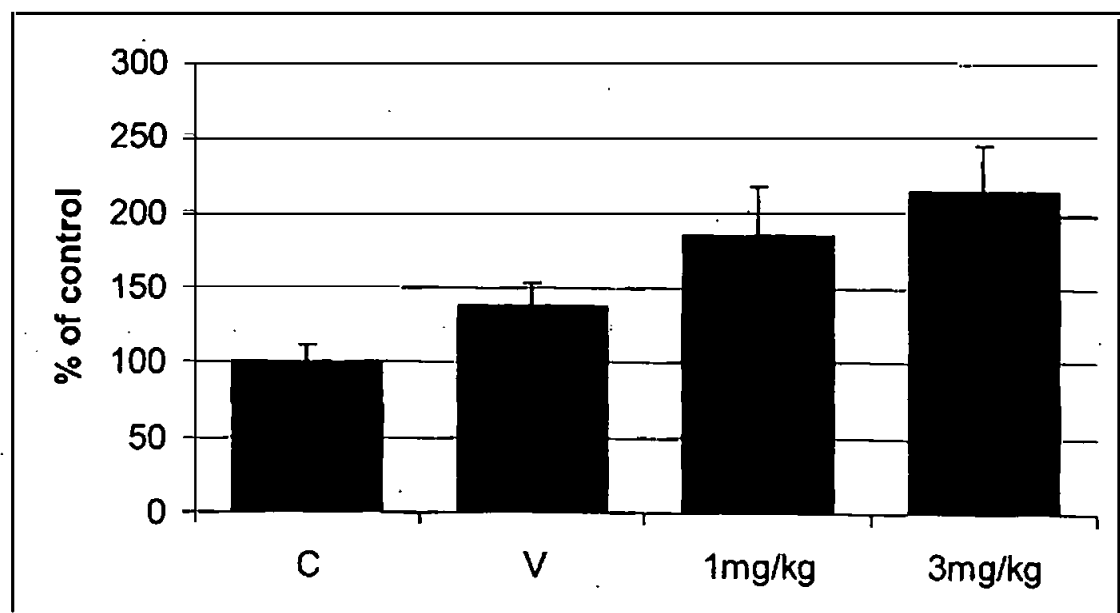


Fig.6



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**INHIBIDORES DE LA PDE Y COMBINACIONES DE LOS MISMOS PARA EL
TRATAMIENTO DE TRASTORNOS UROLÓGICOS**

Campo técnico de la invención

- 5 La presente invención se refiere a fosfodiesterasas (PDE) y la farmacología de los inhibidores de las PDE. Más particularmente, la invención se refiere a inhibidores de la PDE-5 y la PDE-4 y su uso para la preparación de medicamentos para el tratamiento de trastornos urológicos.

Antecedentes de la invención

- 10 La hiperplasia prostática benigna (HPB) que tiene como resultado la obstrucción del tracto de salida de la vejiga (OSV) es una neoplasia muy frecuente en varones. Se ha estimado que aproximadamente el 80% de los varones mayores de 50 años presentan síntomas de moderados a graves, incluidos aumento de frecuencia urinaria, nicturia y urgencia, acompañados de
- 15 un lento chorro urinario y de retención urinaria. Por tanto, la HPB se reconoce cada vez más como un problema importante de salud en los países occidentalizados (Guess, 1995). Además de la cirugía prostática (20% de todos los pacientes con HPB), el tratamiento habitual de la enfermedad comprende inhibidores de la 5-alfa reductasa (finasterida) y alfa bloqueantes (tamsulosina,
- 20 doxazosina, terazosina, alfuzosina) (Truss 2001). Los inhibidores de la 5-alfa reductasa influyen sobre el componente mecánico de la HPB e inhiben la proliferación del tejido prostático. Los alfa bloqueantes influyen sobre el componente dinámico y disminuyen los síntomas irritativos de la HPB mediante la relajación del músculo liso prostático, que disminuye la resistencia de la
- 25 uretra. Además, los alfa bloqueantes son capaces de relajar directamente las

células del músculo liso de la vejiga y reducen las contracciones de no vaciado de la vejiga. No obstante, todas estas opciones de tratamiento tienen una eficacia limitada y/o un perfil desfavorable de efectos secundarios (Carbone, 2003). Por tanto, diversos intentos se han centrado en nuevas opciones
5 terapéuticas para inhibir la proliferación del estroma prostático o disminuir el tono del músculo liso de la próstata y la vejiga. Esto incluye, en concreto, inhibidores de la aromatasa (Sciarra, 2000), antagonistas del factor de crecimiento (Desgrandchamps, 1997), agentes de apertura de los canales de potasio (Gopalakrishnan, 2004) y antagonistas de la endotelina (Andersson,
10 2002).

También está bien establecido que los nucleótidos cíclicos AMPc y GMPc pueden reducir el tono del músculo liso (Drescher, 1994). El AMPc y el GMPc se sintetizan a partir de sus correspondientes nucleósido trifosfatos mediante la adenilato y la guanilato ciclase respectivamente. Se degradan
15 mediante la acción de las fosfodiesterasas de nucleótidos cíclicos (PDE), que regulan los niveles intracelulares de AMPc y GMPc con mucha eficacia. Hasta ahora se han identificado 11 miembros diferentes de la familia de las PDE, que difieren en su estructura, regulación y especificidad por su sustrato (Soderling, 2000). El papel de las PDE en el tratamiento de trastornos urológicos es muy
20 poco conocido, la caracterización de isoformas de las PDE está muy por detrás de otros sistemas y gran parte de la bibliografía se publicó antes de la identificación de las PDE recientemente identificadas. Aunque las PDE se expresan en el tracto urinario inferior, es decir en la vejiga, la uretra y los tejidos prostáticos, todavía no se dispone de datos de la expresión del ARNm y de
25 comparaciones directas de todos los isógenos de las PDE o los disponibles son

incosistentes. Existen algunas pruebas de que la inhibición inespecífica de las PDE es capaz de relajar el tejido prostático humano (Drescher, 1994). Los datos sobre el efecto de la inhibición de la PDE-5 son muy limitados. Se ha mostrado que Zaprinast, un inhibidor de la PDE-5 que también inhibe las PDE-6, -9 y -11, es capaz de relajar in vitro tejido prostático humano pre-contraído (Ückert, 2001), mientras que el papel de otras familias de PDE dentro de este tejido queda todavía por determinar. Dentro de la vejiga, el bloqueo inespecífico de diferentes PDE por IBMX (inhibición de las PDE-1, -2, -3, -4, -5, -6, -10 y -11) podría relajar la vejiga de cobayas hembra, mientras que Zaprinast fue ineficaz (Gillespie, 2004). A pesar de estos hallazgos incosistentes, el papel de la PDE-5 en la relajación del cuerpo cavernoso y el tratamiento de la disfunción eréctil es bien conocido y en el mercado ya hay inhibidores de la PDE-5 muy potentes y selectivos. Inhibidores potentes y selectivos de la PDE-4 se usan principalmente para el tratamiento del asma y la EPOC (Spina, 2003).

15 **Descripción de la invención**

Un aspecto de la invención se proporciona mediante el perfil de expresión del ARNm de la PDE, que demuestra la abundancia de PDE-5 dependiente de GMPc y de PDE-4 dependiente de AMPc, no sólo en la próstata sino también en el tejido vesical (Figuras 1, 2). Por tanto, los inhibidores selectivos de la PDE-5 o la PDE-4, y en particular combinaciones de ambos, no sólo deben reducir la contractilidad prostática sino también, como beneficio adicional de una combinación de ambos, atenuar los síntomas irritativos causados por la obstrucción del tracto de salida de la vejiga como ocurre con frecuencia en los trastornos urológicos. Los inhibidores selectivos de la PDE-5 son, por ejemplo, vardenafilo, sildenafil y tadalafilo, un inhibidor

selectivo de la PDE-4 es, por ejemplo, Roflumilast.

Los trastornos urológicos a los que está dirigidos los agentes terapéuticos de la invención comprenden hiperplasia prostática benigna (HPB), síntomas del tracto urinario inferior (STUI) y, en particular, síntomas irritativos causados por la obstrucción del tracto de salida vesical (OSV) inducida por HPB. Dado que el tratamiento con inhibidores específicos de la PDE-5 y/o la PDE-4 (y, en particular, combinaciones de los mismos) no sólo está dirigido a las irritaciones sintomáticas de la vejiga sino también a las obstrucciones del tracto de salida vesical inducidas por HPB subyacentes, este tratamiento proporciona una considerable ventaja sobre los procedimientos de tratamiento ya conocidos en la técnica.

Otros trastornos urológicos que en particular, y con una considerable ventaja, se pueden tratar mediante los inhibidores mencionados antes, o combinaciones de los inhibidores, son trastornos genitourinarios que comprenden el síndrome vesical neurogénico [también conocido como vejiga hiperactiva (VHA) o cistitis intersticial (CI)], incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas y disfunción eréctil.

Otro aspecto de la invención es la demostración de que el inhibidor de la

PDE-5 vardenafilo tiene un efecto relajante sobre los anillos uretrales de rata con un valor de CE_{50} de 0,96 $\mu\text{mol/l}$, y tiras de próstata y de vejiga de ratas con el valor de CE_{50} de 1,1 y 5,0 $\mu\text{mol/l}$, respectivamente (Figura 3, Tabla 1).

Otro aspecto de la invención es la demostración de que el inhibidor de la PDE-4 Roflumilast y el inhibidor de la PDE-5 vardenafilo muestran ambos efectos de relajación en tiras de vejiga de conejo con una CI_{50} de 260 nmol/l y 1,7 $\mu\text{mol/l}$ respectivamente (Figura 4, Tabla 2).

Otro aspecto de la invención se proporciona mediante la demostración de que el inhibidor de la PDE-5 vardenafilo disminuía significativamente el número de contracciones de no vaciado como medida de síntomas irritativos de la HPB en el modelo de obstrucción del tracto de salida vesical (OSV) de ratas (Figura 5).

La invención proporciona inhibidores de la PDE-5 que, solos o en combinación con inhibidores de la PDE-4, son útiles para el tratamiento de trastornos urológicos. En particular, compuestos de la invención son *Tadalafil* ((6R, 12aR)-2,3,6,7,12,12a-hexahidro-2-metil-6-(3,4-metilendioxfenil)pirazino(1',2':1,6)pirido(3,4-b)-indol-1,4-diona), *Vardenafilo* (2-(2-etoxi-5-(4-etilpiperazin-1-il-1-sulfonil)fenil)-5-metil-7-propil-3H-imidazo (5,1-f)(1,2,4)triazin-4-ona), *Sildenafil* (3-[2-etoxi-5-(4-metilpiperazin-1-il)sulfonilfenil]-7-metil-9-propil-1,2,4,7,8-tetrabíciclo[4.3.0]nona-3,8,10-trien-5-ona), *Udenafilo* 5-[2-propiloxi-5-(1-metil-2-pirrolidiniletílamidosulfonil)fenil]-metil-3-propil-1,6-dihidro-7H-pirazolo(4,3-d)pirimidin-7-ona, *Dasanafilo* 7-(3-bromo-4-metoxibencil)-1-etil-8-[[[(1,2)-2-hidroxíciclopentil]amino]-3-(2-hidroxi-etil)-3,7-dihidro-1-purin-2,6-diona, *Avanafilo* 4-[[[(3-cloro-4-metoxifenil)metil]amino]-2-[(2S)-2-(hidroximetil)pirrolidin-1-il]-N-(pirimidin-2-ilmetil)pirimidin-5-

carboxamida, SLx 2101 de Surface Logix, LAS 34179 triazolo[1,2-]xantina-6-metil-4-propil-2-[2-propoxi-5-(4-metilpiperzin)sulfonil]fenilo, Roflumilast (3-(ciclopropilmetoxi)-N-(3,5-dicloropiridin-4-il)-4-(difluorometoxi)benzamida), Cilomilast ácido (4-ciano-4-(3-ciclopentoxi-4-metoxi-fenil)-ciclohexano-1-carboxílico y Piclamilast (3-ciclopentoxi-N-(3,5-dicloropiridin-4-il)-4-metoxi-benzamida).

Otro aspecto más de la invención es un procedimiento de detección selectiva de inhibidores de la PDE, en particular de inhibidores de la PDE-4 y la PDE-5, para usar, solos o en combinación, para la preparación de medicamentos para el tratamiento de los trastornos urológicos mencionados antes.

La invención proporciona procedimientos (también denominados en la presente memoria descriptiva "ensayos de detección selectiva") para identificar inhibidores de la PDE que se pueden usar para el tratamiento de trastornos urológicos. Los procedimientos implican la identificación de compuestos o agentes candidatos o de prueba (p. ej., péptidos, peptidomiméticos, moléculas pequeñas u otras moléculas) que se unen a las fosfodiesterasas y/o tienen un efecto estimulador o inhibidor sobre la actividad biológica de la PDE1A o su expresión y, después, la determinación de cuál de estos compuestos tiene un efecto sobre los síntomas o enfermedades relacionados con trastornos urológicos en un ensayo in vivo.

Compuestos o agentes candidatos o de prueba que se unen a la PDE-4 o la PDE-5 y/o tienen un efecto estimulador o inhibidor de la actividad o la expresión de la PDE-4 o la PDE-5 se identifican bien en ensayos que emplean células que expresan PDE-4 y/o PDE-5 (ensayos basados en células) o bien en

- ensayos con PDE-4 y/o PDE-5 aisladas (ensayos sin células). Los diversos ensayos pueden emplear diversas variantes de PDE (p. ej., PDE de longitud completa, un fragmento biológicamente activo de las PDE, o una proteína de fusión que incluya toda o una porción de PDE). Además, la PDE-4 y/o la PDE-5
- 5 pueden derivar de cualquier especie de mamífero adecuada. El ensayo puede ser un ensayo de unión que implique la medición directa o indirecta de la unión de un compuesto de prueba o un ligando conocido de la PDE-4 o la PDE-5 a la PDE-4 o la PDE-5. El ensayo también puede ser un ensayo de actividad que implique la medición directa o indirecta de la actividad de la PDE-4 o la PDE-5.
- 10 El ensayo también puede ser un ensayo de expresión que implique la medición directa o indirecta de la expresión de ARNm de PDE-4 y/o PDE-5 o proteína de PDE-4 y/o PDE-5. Los diversos ensayos de detección selectiva se combinan con un ensayo in vivo que implique medir el efecto del compuesto de prueba sobre los síntomas de trastornos urológicos.
- 15 La presente invención incluye ensayos bioquímicos sin células que permiten la identificación de inhibidores y agonistas de PDE adecuados como estructuras líder para el desarrollo farmacológico del fármaco. Tales ensayos implican poner en contacto la PDE-4 y/o la PDE-5 con un compuesto de prueba y determinar la capacidad del compuesto de prueba para actuar como
- 20 antagonista (preferentemente) o un agonista de la actividad enzimática de la PDE-4 y/o la PDE-5. En una forma de realización, el ensayo incluye la el control de la actividad PDE de la PDE-4 y/o la PDE-5 midiendo la conversión del AMPc o el GMPc en su nucleósido monofosfato tras el contacto de la PDE-4 y/o la PDE-5 con un compuesto de prueba.
- 25 Por ejemplo, los niveles de AMPc y de GMPc se pueden medir mediante

el uso de compuestos que contengan tritio 3HAMPc y 3HGMPc como se describe en [Hansen, R.S. y Beavo, J.A., PITAS, USA 1982, 79: 2788-92]. Para seleccionar un grupo de compuestos comprendido por un gran número de compuestos se puede aplicar el ensayo de proximidad por centelleo basado en
5 placas de microtitulación (SPA) como se describe en [Bardelle, C. y col. (1999) Anal. Biochem. 275: 148-155].

Como alternativa, se puede analizar la actividad fosfodiesterasa de la proteína recombinante usando un kit de SPA disponible comercialmente (Amersham Pharmacia). La enzima PDE hidroliza los nucleótidos cíclicos, por
10 ejemplo AMPc y GMPc, en sus homólogos lineales. El ensayo SPA utiliza los nucleótidos cíclicos tritiados [3H]AMPc o [3H]GMPc, y se basa en la interacción selectiva del producto no cíclico tritiado con las perlas del SPA, mientras que los sustratos cíclicos no se unen con eficacia.

El producto radiomarcado unido a las perlas de centelleo genera luz que
15 se puede analizar en un contador de centelleo.

Se formula una composición farmacéutica de la invención para que sea compatible con su vía de administración pretendida. Ejemplos de vías de administración incluyen las vías parenteral (p. ej., administración intravenosa, intraarterial, intradérmica, subcutánea, intramuscular, inhaladora, transdérmica,
20 transmucosa, nasal y rectal), oral (p. ej., administración bucal, sublingual, mucosa oral y perioral) y local (p. ej., instilación local de soluciones o suspensiones e implantes locales).

Entre las composiciones farmacéuticas adecuadas para inyecciones e infusiones se incluyen soluciones acuosas estériles (si el ingrediente activo es
25 lo bastante soluble en agua), suspensiones, emulsiones y polvos estériles para

la preparación extemporánea de soluciones inyectables estériles o dispersiones. El portador puede ser un medio disolvente o de dispersión que contenga, por ejemplo, agua, etanol, un poliol farmacéuticamente aceptable como glicerol, propilenglicol, polietilenglicol líquido, y mezclas adecuadas de los mismos. Se pueden añadir ingredientes farmacéuticamente aceptables como tampones, conservantes, antioxidantes, agentes isotonizantes o tensioactivos. Las inyecciones de liberación prolongada se basan en principios de formulaciones conocidas como soluciones oleosas o suspensiones o partículas de polímeros biodegradables.

- 10 Para la administración mediante inhalación, los compuestos se liberan en forma de un pulverizador de aerosoles desde un contenedor o dispensador presurizado que contiene un propelente adecuado desde un nebulizador o un inhalador en polvo seco.

La administración sistémica también puede ser por medios transmucoso o transdérmico. Para la administración transmucosa o transdérmica, en la formulación se usan penetrantes adecuados para la barrera a permear. Tales penetrantes son, en general, conocidos en la técnica e incluyen, por ejemplo, para administración transmucosa, detergentes, sales biliares y derivados de ácido fusídico. La administración transmucosa se puede conseguir a través del uso de pulverizadores nasales, preparaciones sublinguales o bucales o supositorios. Para la administración transdérmica, los compuestos activos se formulan en ungüentos, parches, geles o cremas tal y como se conocen generalmente en la técnica.

- 25 Los compuestos también se pueden preparar en forma de supositorios (p. ej., con bases de supositorio convencionales tales como manteca de cacao

y otros glicéridos) o enemas de retención para liberación rectal.

Generalmente, las composiciones orales incluyen un diluyente inerte o agente de carga y excipientes funcionales. Pueden introducirse en cápsulas o comprimirse en comprimidos. Otras formas de dosificación adecuadas son
5 comprimidos efervescentes, comprimidos masticables o comprimidos orodispersables, cápsulas de gelatina blanda, cápsulas de gelatina dura cargadas con líquido, polvos en sellos y líquidos orales.

Excipientes funcionales adecuados para la preparación de formas de dosificación oral son bien conocidos en la técnica e incluyen, por ejemplo,
10 ligantes tales como polivinilpirrolidona o hidroxipropilmetilcelulosa, disgregantes tales como crospovidona o croscarmelosa sódica, deslizantes tales como dióxido de silicio coloidal, lubricantes tales como estearato de magnesio, macrogoles o ácido esteárico, agentes edulcorantes tales como aspartamo, sacarosa o sacarina sódica y agentes aromatizantes tales como aroma a menta
15 o a naranja.

En una forma de realización, los compuestos activos se preparan con portadores que protegerán el compuesto contra su rápida eliminación del cuerpo, tales como comprimidos de liberación controlada o esferas recubiertas cargadas en cápsulas o formulaciones parenterales de liberación controlada,
20 incluidos implantes y sistemas de liberación microencapsulados. Se pueden usar polímeros biodegradables o biocompatibles, tales como etilen vinil acetato, polianhidridos, ácido poliglicólico, colágeno, poliortoésteres, ácido poliláctico o copolímeros poliglicólico-poliláctico.

En otra forma de realización, la invención proporciona combinaciones de
25 inhibidores de la PDE-4 y la PDE-5 y su uso para la preparación de

composiciones farmacéuticas para el tratamiento de trastornos urológicos, de modo que estas combinaciones comprenden bien i) composiciones farmacéuticas que comprenden un compuesto que posee acción inhibidora de tanto la actividad PDE-4 como la PDE-5, o ii) composiciones farmacéuticas que
5 comprenden al menos un inhibidor de la PDE-4 y al menos un inhibidor de la PDE-5 como una combinación fija en una unidad de aplicación, o iii) un kit de partes que contiene al menos dos grupos de composiciones farmacéuticas, donde cada grupo consta de al menos una preparación farmacéutica que comprende un inhibidor de la PDE-5 en unidades de al menos una dosis y al
10 menos una preparación farmacéutica que comprende un inhibidor de la PDE-4 en unidades de al menos una dosis, donde cada unidad de aplicación de dichas composiciones farmacéuticas se administra en combinación, secuencialmente, como dosis única o en múltiples dosis.

La presente invención proporciona además:

15 Un procedimiento de detección selectiva de inhibidores de la PDE-5 útiles como agentes terapéuticos en el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), que comprende las etapas de i)
20 poner en contacto un compuesto de prueba (que puede o no tener actividad inhibidora de la PDE-4) con un polipéptido de PDE-5, ii) determinar la actividad del polipéptido de PDE5 a cierta concentración del compuesto de prueba o en ausencia de dicho compuesto de prueba, iii) determinar la actividad de dicho polipéptido de PDE5 a una concentración diferente de dicho compuesto de
25 prueba, iv) seleccionar al menos un compuesto con efecto inhibidor sobre el

polipéptido de PDE-5.

Un procedimiento para la detección selectiva de inhibidores de la PDE-4 útiles como agentes terapéuticos en el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia

5 prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), que comprende las etapas de i) poner en contacto un compuesto de prueba (que puede o no tener actividad inhibidora de la PDE-5) con un polipéptido de PDE-4, ii) determinar la actividad del polipéptido de PDE4 a cierta concentración del compuesto de prueba o en

10 ausencia de dicho compuesto de prueba, iii) determinar la actividad de dicho polipéptido de PDE4 a una concentración diferente de dicho compuesto de prueba, iv) seleccionar al menos un compuesto con efecto inhibidor sobre el polipéptido de PDE-4.

Un procedimiento para la detección selectiva de combinaciones de

15 inhibidores de la PDE-4 y de la PDE -5 útiles como agentes terapéuticos en el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), que comprende las etapas de i) poner en contacto un primer compuesto de prueba

20 con un polipéptido de PDE-5, ii) determinar la actividad del polipéptido PDE5 a cierta concentración del primer compuesto de prueba o en ausencia de dicho primer compuesto de prueba, iii) determinar la actividad de del polipéptido PDE5 a una concentración diferente de dicho primer compuesto de prueba, iv) seleccionar al menos un primer compuesto con efecto inhibidor sobre el

25 polipéptido de PDE-5, v) poner en contacto un segundo compuesto de prueba

- con un polipéptido de PDE-4, vi) determinar la actividad del polipéptido de PDE4 a cierta concentración del segundo compuesto de prueba o en ausencia de dicho segundo compuesto de prueba, vii) determinar la actividad del polipéptido de PDE4 a una concentración diferente de dicho segundo compuesto de prueba, vii) seleccionar al menos un segundo compuesto con efecto inhibidor sobre el polipéptido de PDE-4, vii) combinar al menos un primer compuesto con actividad inhibidora de la PDE5 con al menos un segundo compuesto que tenga actividad inhibidora de PDE4.

- Procedimientos de detección selectiva que implican poner en contacto el compuesto de prueba dentro o en la superficie de una célula, en los que la célula está in vitro.

Procedimientos de detección selectiva que implican poner en contacto el compuesto de prueba con el polipéptido de PDE-4 o PDE-5 en un sistema sin células.

- Los procedimientos de detección selectiva pueden implicar un compuesto de prueba que esté acoplado a un marcador detectable.

En particular, la presente invención proporciona:

- Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV), síntomas del tracto urinario inferior (STUI), trastornos genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por reflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres,

enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica y uropatías obstructivas en un
5 mamífero, que comprende un agente terapéutico que regula la actividad de un polipéptido de PDE5.

Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y
10 síntomas del tracto urinario inferior (STUI) en un mamífero, que comprende un agente terapéutico que regula la actividad de un polipéptido de PDE4.

Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y
15 síntomas del tracto urinario inferior (STUI) en un mamífero, que comprende un agente terapéutico que es una combinación de los agentes terapéuticos selectivos mencionados anteriormente.

Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y
20 síntomas del tracto urinario inferior (STUI) en un mamífero, que comprende un agente terapéutico que regula la actividad de un polipéptido de PDE5 y de un polipéptido de PDE4.

Una composición farmacéutica para el tratamiento de una enfermedad
25 comprendida en un grupo de enfermedades constituidas por hiperplasia

prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero, que comprende un inhibidor de la PDE-4 seleccionado del grupo de inhibidores de la PDE-4 compuesto por Roflumilast (3-(ciclopropilmetoxi)-N-(3,5-dicloropiridin-4-il)-4-
 5 (difluorometoxi)benzamida), Cilomilast ácido (4-ciano-4-(3-ciclopentoxi-4-metoxi-fenil)-ciclohexano-1-carboxílico) y Piclamilast (3-ciclopentoxi-N-(3,5-dicloropiridin-4-il)-4-metoxi-benzamida).

Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia
 10 prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), trastornos genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los
 15 órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica y uropatías obstructivas en un
 20 mamífero, que comprende un inhibidor de la PDE-5 seleccionado del grupo de inhibidores de la PDE-5 compuesto por *Tadalafilo* ((6R, 12aR)-2,3,6,7,12,12a-hexahidro-2-metil-6-(3,4-metilen-dioxifenil)pirazino(1',2':1,6)pirido(3,4-b)-indol-1,4-diona), *Vardenafilo* (2-(2-etoxi-5-(4-etilpiperazin-1-il-1-sulfonil)fenil)-5-metil-7-propil-3H-imidazo (5,1-f)(1,2,4)triazin-4-ona), *Sildenafil* (3-[2-etoxi-5-(4-
 25 metilpiperazin-1-il)sulfonil-fenil]-7-metil-9-propil-1,2,4,7,8-tetrabíciclo[4.3.0]nona-

3,8,10-trien-5-ona), *Udenafilo* 5-[2-propiloxi-5-(1-metil-2-pirrolidiniletilamidossulfonil)fenil]-metil-3-propil-1,6-dihidro-7H-pirazolo(4,3-d)pirimidin-7-ona, *Dasantafilo* 7-(3-bromo-4-metoxibencil)-1-etil-8-[[[(1,2)-2-hidroxiciclopentil]amino]-3-(2-hidroxietil)-3,7-dihidro-1-purin-2,6-diona, *Avanafilo*
5 4-[[[(3-cloro-4-metoxifenil)metil]amino]-2-[(2S)-2-(hidroximetil)pirrolidin-1-il]-N-(pirimidin-2-ilmetil)pirimidin-5-carboxamida, *SLx 2101* de Surface Logix, *LAS 34179* triazolo[1,2-xantina-6-metil-4-propil-2-[2-propoxi-5-(4-metilpiperzin)sulfonil]fenilo, o sales, hidratos o hidratos de sales de los mismos.

Una composición farmacéutica para el tratamiento de una enfermedad
10 comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), en un mamífero, que comprende una combinación de al menos un inhibidor de la PDE-4 seleccionado del grupo de inhibidores de la PDE-4 compuesto por Roflumilast (3-(ciclopropilmetoxi)-N-
15 (3,5-dicloropiridin-4-il)-4-(difluorometoxi)benzamida), Cilomilast ácido (4-ciano-4-(3-ciclopentoxi-4-metoxi-fenil)-ciclohexano-1-carboxílico) y Piclamilast (3-ciclopentoxi-N-(3,5-dicloropiridin-4-il)-4-metoxi-benzamida, y al menos un inhibidor de la PDE-5 seleccionado del grupo de inhibidores de la PDE-5 compuesto por Vardenafilo (2-(2-etoxi-5-(4-etilpiperazin-1-il-1-sulfonil)fenil)-5-
20 metil-7-propil-3H-imidazo (5,1-f)(1,2,4)triazin-4-ona), Sildenafil (3-[2-etoxi-5-(4-metilpiperazin-1-il)sulfonil-fenil]-7-metil-9-propil-1,2,4,7,8-tetrabicciclo[4.3.0]nona-3,8,10-trien-5-ona) y Tadalafilo ((6R, 12aR)-2,3,6,7,12,12a-hexahidro-2-metil-6-(3,4-metilen-dioxifenilo).

Uso de un inhibidor de la PDE5 para la preparación de una composición
25 farmacéutica para el tratamiento de una enfermedad comprendida en un grupo

de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), trastornos genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por reflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica y uropatías obstructivas en un mamífero.

Uso de un inhibidor de la PDE4 para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero.

Uso de una combinación de al menos un inhibidor de la PDE4 y al menos un inhibidor de la PDE5 para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), trastornos genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por reflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema

genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, 5 uropatías obstructivas y disfunción eréctil en un mamífero.

Uso de un agente que es un inhibidor de un polipéptido de PDE4 y un polipéptido de PDE5 para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), 10 obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero.

Uso de un inhibidor de la PDE-5 seleccionado del grupo de inhibidores de la PDE-5 compuesto por *Tadalafilo* ((6R, 12aR)-2,3,6,7,12,12a-hexahidro-2-metil-6-(3,4-metilen-dioxifenil)pirazino(1',2':1,6)pirido(3,4-b)-indol-1,4-diona), 15 *Vardenafilo* (2-(2-etoxi-5-(4-etilpiperazin-1-il-1-sulfonil)fenil)-5-metil-7-propil-3H-imidazo (5,1-f)(1,2,4)triazin-4-ona), *Sildenafil* (3-[2-etoxi-5-(4-metilpiperazin-1-il)sulfonil-fenil]-7-metil-9-propil-1,2,4,7,8-tetrabíciclo[4.3.0]nona-3,8,10-trien-5-ona), *Udenafilo* 5-[2-propiloxi-5-(1-metil-2-pirrolidiniletilamidossulfonil)fenil]-metil-3-propil-1,6-dihidro-7H-pirazolo(4,3-d)pirimidin-7-ona, *Dasatafilo* 7-(3-bromo-4-metoxibencil)-1-etil-8-[[[(1,2)-2-hidroxíciclopentil]amino]-3-(2-hidroxietil)-3,7-dihidro-1-purin-2,6-diona, *Avanafilo* 4-[[[(3-cloro-4-metoxifenil)metil]amino]-2-[(2S)-2-(hidroximetil)pirrolidin-1-il]-N-(pirimidin-2-ilmetil)pirimidin-5-carboxamida, *SLx 2101* de Surface Logix, *LAS 34179* triazolo[1,2-]xantina-6-metil-4-propil-2-[2-propoxi-5-(4-metilpiperzin)sulfonil]fenilo, o sales, hidratos o 25 hidratos de sales de los mismos, para la preparación de una composición

farmacéutica para el para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), trastornos genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica y uropatías obstructivas en un mamífero.

Uso de un inhibidor de la PDE-4 seleccionado del grupo de inhibidores de la PDE-4 compuesto por Roflumilast (3-(ciclopropilmetoxi)-N-(3,5-dicloropiridin-4-il)-4-(difluorometoxi)benzamida), Cilomilast ácido (4-ciano-4-(3-ciclopentoxi-4-metoxi-fenil)-ciclohexano-1-carboxílico y Piclamilast (3-ciclopentoxi-N-(3,5-dicloropiridin-4-il)-4-metoxi-benzamida), para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero.

Uso de una combinación de al menos un inhibidor de la PDE-4 seleccionado del grupo de inhibidores de la PDE-4 compuesto por Roflumilast (3-(ciclopropilmetoxi)-N-(3,5-dicloropiridin-4-il)-4-(difluorometoxi)benzamida),

Cilomilast ácido (4-ciano-4-(3-ciclopentoxi-4-metoxi-fenil)-ciclohexano-1-carboxílico y Piclamilast (3-ciclopentoxi-N-(3,5-dicloropiridin-4-il)-4-metoxi-benzamida) y al menos un inhibidor de la PDE-5 seleccionado del grupo de inhibidores de la PDE-5 compuesto por Vardenafilo (2-(2-etoxi-5-(4-etilpiperazin-1-il-1-sulfonil)fenil)-5-metil-7-propil-3H-imidazo (5,1-f)(1,2,4)triazin-4-ona), Sildenafil (3-[2-etoxi-5-(4-metilpiperazin-1-il)sulfonil-fenil]-7-metil-9-propil-1,2,4,7,8-tetrabicyclo[4.3.0]nona-3,8,10-trien-5-ona) y Tadalafilo ((6R, 12aR)-2,3,6,7,12,12a-hexahidro-2-metil-6-(3,4-metilen-dioxifenilo), para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), trastornos genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas y disfunción eréctil en un mamífero.

Un procedimiento para la preparación de una composición farmacéutica útil para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario

inferior (STUI), trastornos genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas y disfunción eréctil en un mamífero, que comprende las etapas de i) identificar un inhibidor de la PDE-5 de acuerdo con el procedimiento de detección selectiva descrito antes, ii) identificar un inhibidor de la PDE4 de acuerdo con el procedimiento de detección selectiva descrito antes, determinar si dichos inhibidores atenúan los síntomas de una enfermedad comprendida en un grupo de enfermedades compuestas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero; e iii) combinar al menos uno de dichos inhibidores con un portador farmacéutico aceptable.

Un procedimiento para la preparación de una composición farmacéutica, en el que el inhibidor de la PDE-5 es un inhibidor de la PDE-5 seleccionado del grupo de inhibidores de la PDE-5 compuesto por Vardenafilo (2-(2-etoxi-5-(4-etilpiperazin-1-il-1-sulfonil)fenil)-5-metil-7-propil-3H-imidazo (5,1-f)(1,2,4)triazin-4-ona), Sildenafil (3-[2-etoxi-5-(4-metilpiperazin-1-il)sulfonil-fenil]-7-metil-9-propil-1,2,4,7,8-tetrabicyclo[4.3.0]nona-3,8,10-trien-5-ona), Tadalafil ((6R, 12aR)-2,3,6,7,12,12a-hexahidro-2-metil-6-(3,4-metilen-dioxifenilo), Udenafilo 5-

[2-propiloxi-5-(1-metil-2-pirrolidiniletilamidossulfonil)fenil]-metil-3-propil-1,6-dihidro-7H-pirazolo(4,3-d)pirimidin-7-ona, Dasantafile 7-(3-bromo-4-metoxibencil)-1-etil-8-[[[(1,2)-2-hidroxiciclopentil]amino]-3-(2-hidroxietil)-3,7-dihidro-1-purin-2,6-diona, Avanafilo 4-[[[(3-cloro-4-metoxifenil)metil]amino]-2-
 5 [(2S)-2-(hidroximetil)pirrolidin-1-il]-N-(pirimidin-2-ilmetil)pirimidin-5-carboxamida, SLx 2101 de Surface Logix, LAS 34179 triazolo[1,2-]xantina-6-metil-4-propil-2-[2-propoxi-5-(4-metilpiperzin)sulfonil]fenilo.

Un procedimiento para la preparación de una composición farmacéutica, en el que el inhibidor de la PDE-4 es un inhibidor de la PDE-4 seleccionado del
 10 grupo de inhibidores de la PDE-4 compuesto por Roflumilast (3-(ciclopropilmetoxi)-N-(3,5-dicloropiridin-4-il)-4-(difluorometoxi)benzamida), Cilomilast ácido (4-ciano-4-(3-ciclopentoxi-4-metoxi-fenil)-ciclohexano-1-carboxílico y Piclamilast (3-ciclopentoxi-N-(3,5-dicloropiridin-4-il)-4-metoxi-benzamida).

15 Uso de una composición farmacéutica como se ha mencionado antes para la regulación de la actividad de la PDE en un mamífero que presenta una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV), síntomas del tracto urinario inferior (STUI), trastornos
 20 genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda,
 25 enfermedades renales mediadas por el sistema inmunológico como rechazo de

trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas y disfunción eréctil.

Un kit de partes para el tratamiento de una enfermedad comprendida en

5 un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), en un mamífero, incluidos seres humanos, que contienen una combinación de al menos una composición farmacéutica seleccionada del grupo de composiciones farmacéuticas compuesto por

10 Vardenafilo, Sildenafil y Tadalafilo, y al menos una composición farmacéutica seleccionada del grupo de composiciones farmacéuticas compuesto por Roflumilast, Cilomilast y Piclamilast.

Un procedimiento para la preparación de un kit de partes útil para el tratamiento de una enfermedad comprendida en un grupo de enfermedades

15 constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), en un mamífero, que comprende las etapas de i) seleccionar al menos una composición farmacéutica del grupo de composiciones farmacéuticas constituido por Vardenafilo, sildenafil y Tadalafilo, ii) seleccionar al menos una

20 composición farmacéutica del grupo de composiciones farmacéuticas compuesto por Roflumilast, Cilomilast y Piclamilast, iii) combinar al menos dos de dichas composiciones farmacéuticas de modo que se crea dicho kit de partes.

Un kit de partes para el tratamiento de una enfermedad comprendida en

25 un grupo de enfermedades constituidas por hiperplasia prostática benigna

(HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), en un mamífero, que contiene una combinación de al menos un agente terapéutico que regula la actividad de un polipéptido de PDE-5 y Tadalafilo y al menos un agente terapéutico que regula la actividad de un polipéptido de PDE-4.

Un procedimiento para la preparación de un kit de partes útil para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), en un mamífero, que comprende las etapas de i) seleccionar al menos una composición farmacéutica que comprende un agente terapéutico que regula la actividad de un polipéptido de PDE-5, ii) seleccionar al menos una composición farmacéutica que comprende un agente terapéutico que regula la actividad de un polipéptido de PDE-4, iii) combinar al menos dos de dichas composiciones farmacéuticas de modo que se crea dicho kit de partes.

Una forma de realización preferida de la invención es una composición farmacéutica que contiene Vardenafilo, o una sal, un hidrato o un hidrato de una sal del mismo, para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV), síntomas del tracto urinario inferior (STUI), trastornos genitourinarios constituidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia

renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas en un mamífero.

- 5 Sorprendentemente se ha encontrado que, especialmente Vardenafilo, o una sal, un hidrato o un hidrato de una sal del mismo, exhibe mayores actividades y muestra mejores resultados en el tratamiento de la vejiga neurogénica (también denominada vejiga hiperactiva o cistitis intersticial) en comparación con otros inhibidores de PDE-5.

10 **Descripción de las figuras**

Figura 1: Expresión relativa de ARNm de PDE-5 en Riñón (K), Vejiga (B), Próstata (P), Uretra (U) y Cuerpo Cavernoso (C) de ratas Sprague Dawley. Los datos son la media + ETM, n= 10.

- Figura 2: Expresión relativa de ARNm de PDE-4a, -4b, -4c, -4d y PDE-5 en
15 vejiga y próstata de ratas Sprague Dawley. Los datos son la media + ETM, n= 10.

- Figura 3: Efectos de vardenafilo sobre la contracción de anillos uretrales de rata aislados (triángulos negros) y tiras de vejiga (rombos negros) y próstata (cuadrados grises). Las tiras de vejiga se contrajeron previamente usando
20 solución de Krebs-Henseleit con K⁺ (50 mmol/l). Los tejidos uretrales y de próstata se contrajeron previamente usando fenilefrina 10 µmol/l. La relajación se expresó en forma de porcentaje de la pre-contracción. Cada punto representa un valor medio ± ETM, n= 9.

- Figura 4: Efectos de roflumilast (rombos negros) y Vardenafilo (cuadrados
25 grises) sobre la contracción de tiras de vejiga de conejo aisladas. Las tiras de

vejiga se contrajeron previamente usando solución de Krebs-Henseleit con K⁺ (50 mmol/l). La relajación se expresó en forma de porcentaje de la pre-contracción. Cada punto representa un valor medio $\pm$ ETM, n= 9.

Figura 5: Número de contracciones de no-vaciado en % tras tratamiento i.v. en bolo de vehículo (V) y Vardenafilo HCl (1, 3 y 10 mg/kg). Los datos son la media + ETM, *= significativo con $p < 0,05$ (prueba t de student pareada).

Figura 6: Intervalo de micción en % tras tratamiento i.v. en bolo de vehículo (V) y Vardenafilo HCl (1,3 mg/kg) en comparación con el intervalo de micción basal (C). Los datos son la media + ETM, *= significativo con $p < 0,05$ (prueba t de student pareada).

Ejemplo 1

Obtención de muestra de tejido y preparación de ARN: Para la recolección de tejido se usaron ratas Sprague Dawley macho con un peso corporal entre 200-250 g. Las ratas se anestesiaron brevemente con una mezcla de isoflurano al 5% (Baxter S.A.) en un portador con 70% de N₂O y 30% de O₂, y después se procedió a la eutanasia mediante decapitación. El abdomen se abrió mediante una incisión en la línea media y se expusieron los riñones y el tejido del tracto urinario inferior como médula renal, los uréteres, la vejiga, la próstata y la uretra y se extirparon rápidamente. Los tejidos se congelaron en N₂ líquido y se almacenaron hasta la preparación del ARN. El ARN total se aisló usando mini columnas RNeasy (Qiagen Inc.) y después se purificó mediante digestión con ADNasa.

Cuantificación del ARNm de las PDE: La expresión del ARNm de los diferentes isogenes de PDE en tejidos de tracto urinario inferior de ratas se midió mediante PCR cuantitativa en tiempo real (TaqMan-PCR, Heid 1996). Por

tanto, se transcribió 1 µg de ARN total en ADNc con un kit de síntesis de ADNc Superscript II RT (Gibco, Inc) de acuerdo con el manual del suministrador. El ARNm de las PDE se midió mediante RT-PCR cuantitativa en tiempo real en un instrumento de detección de secuencia ABI Prism 7700 (Applied Biosystems, Inc.). Mediante el software Primer Express 1.5 (Applied Biosystems Inc.) se diseñaron secuencias específicas para cebadores directos e inversos así como para la sonda fluorogénica del ARNm del isogen de cada PDE. Durante la amplificación por PCR, la actividad 5' nucleolítica de la Taq polimerasa escinde la sonda separando el tinte fluorescente indicador en 5' del tinte inactivador en 3'. El ciclo umbral, Ct, que se correlaciona inversamente con el nivel diana de ARNm, se midió como el número de ciclo en el cual la emisión fluorescente del indicador aumenta 10 desviaciones típicas por encima del nivel básico. Como gen doméstico, la acción beta se cuantificó como se ha descrito antes, usando como cebador directo 5'-accttcaacaccccagcca-3', cebador inverso 5'-cagtggtacgaccagaggca-3' y sonda fluorescente 5'-6AFM-acgtagccatccaggctgtgtgtcc-TAMARA-3'. Los niveles de ARNm de PDE se corrigieron para los niveles de ARNm de beta-actina y se calcularon como la expresión relativa usando el método comparativo Ct.

Expresión de ARNm de PDE-5 y PDE-4A, -4B, -4C, -4D en el tracto urinario inferior: Dado que sólo se dispone de datos incompletos sobre el perfil de expresión de la PDE-5 en el tejido del tracto urinario inferior se cuantificó el ARNm de PDE en ratas Sprague Dawley macho mediante Taqman RT-PCR. La expresión más predominante de la PDE-5 se encontró en la vejiga (Figura 1). Los niveles de expresión inferiores se encontraron en la uretra, el cuerpo cavernoso y la próstata (Figura 1). Estos resultados mostraron que existe una

considerable expresión de ARNm de la PDE-5 en tejidos del tracto urinario inferior, especialmente en la vejiga.

Además, la expresión de ARNm de la PDE-4 de los cuatro isógenos de la PDE-4 (PDE-4A, -4B, -4C y -4D) se determinó mediante Taqman RT-PCR en la vejiga y en la próstata (Figura 2). Los autores encontraron una expresión muy baja de la PDE-4c, que estaba en el límite de la detectabilidad, no obstante los ARNm de la PDE-4A, -4B Y -4D se expresaron sustancialmente en ambos tejidos. En la vejiga, la PDE-4D fue el ARNm del isógeno de PDE-4 más abundante, mientras que en la próstata la PDE-4A y -4D están casi igualmente distribuidas y se expresan 2,5 veces más que el ARNm de la PDE-5 (Figura 2).

El perfil de expresión demuestra que los ARNm de las PDE-5 y PDE-4D son abundantes en la vejiga, pero también en el tejido prostático. Por tanto, los inhibidores de la PDE-5 o PDE-4, aunque en particular combinaciones de ambos, inhibidores de la PDE-5 y la PDE-4, tales como vardenafilo en combinación con Roflumilast, deberían reducir la contractilidad no sólo en la vejiga sino también en la próstata, lo que proporciona una ventaja sobre procedimientos de tratamiento para trastornos urológicos ya existentes en la técnica actual, donde dichos trastornos comprenden, por ejemplo, hiperplasia prostática benigna (HPB) y, en particular, síntomas irritativos causados por la obstrucción del tracto de salida de la vejiga (OSV) inducida por la HPB, incluidos, entre otros, síntomas del tracto urinario inferior (STUI).

Ejemplo 2

Preparación de tejido: Se procedió a la eutanasia de ratas Wistar macho (200-300 g) usando dióxido de carbono. Los tejidos se extrajeron y se colocaron en tampón de Krebs-Henseleit enfriados con hielo de la siguiente composición (en

mmol/l): NaCl 112, KCl 5,9, CaCl₂ 2,0, MgCl₂ 1,2, NaH₂PO₄ 1,2, NaHCO₃ 25, glucosa 11,5. Del cuerpo de la vejiga se cortaron cuatro tiras longitudinales de igual tamaño de aproximadamente 2 mm x 10 mm. Tiras de próstata se obtuvieron cortando transversalmente a través de los lóbulos de la glándula
5 prostática en paralelo a la uretra. De la parte proximal de la uretra se diseccionó un anillo por animal.

Conejos blancos New Zealand se anestesiaron usando tiopental. Se extrajo la vejiga urinaria y se colocó en tampón de Krebs-Henseleit enfriado con hielo de la siguiente composición (en mmol/l): NaCl 112, KCl 5,9, CaCl₂ 2,0,
10 MgCl₂ 1,2, NaH₂PO₄ 1,2, NaHCO₃ 25 y glucosa 11,5. Del cuerpo de la vejiga se cortaron tiras longitudinales de igual tamaño de aproximadamente 2 mm x 10 mm.

Registro de la actividad mecánica: Las preparaciones se transfirieron a baños del órgano de 20 ml que contiene solución de Krebs-Henseleit
15 equilibrada con O₂ al 95%, CO₂ al 5% a 37°C. Las tiras se montaron entre dos ganchos por medio de dos clip. Para registrar la tensión isométrica, uno de los ganchos se conectó a un transductor de fuerza que a su vez estaba unido a un amplificador y registrador de gráficos. El otro gancho estaba unido a una unidad móvil, lo que permite un ajuste preciso de la tensión de precarga. A
20 continuación se dio a todos los tejidos un periodo de equilibrado de 60 min, durante los cuales se lavaron y la tensión de reposo se ajustó a 1 g cada 20 min.

Tras el periodo de equilibrado, cada experimento se inició exponiendo la preparación a solución de Krebs-Henseleit K⁺ (50 mmol/l). El procedimiento se
25 repitió 3 veces y los tejidos se lavaron al menos tres veces entre cada

contracción.

A continuación, las tiras de vejiga se contrajeron previamente usando solución de Krebs-Henseleit K⁺ (50 mmol/l). Cuando la contracción se estabilizó se construyó una curva de respuesta a la dosis acumulativa del compuesto analizado. La contracción estabilizada inducida por la solución de 5 Krebs-Henseleit K⁺ (50 mmol/l) se definió como una tensión del 100%. La relajación se expresó en forma de tensión en porcentaje.

Tiras prostáticas y anillos uretrales se contrajeron previamente usando 10⁻⁶ mol/l de fenilefrina. Los efectos de los compuestos sobre el tejido 10 prostático se analizaron de un modo no acumulativo con etapas de lavado entre cada concentración.

Ensayo de baño de órgano: Efectos de vardenafilo sobre órganos urogenitales de rata aislados: Los efectos del inhibidor de la PDE5 vardenafilo sobre la relajación de los músculos lisos se analizaron en el sistema del baño de 15 órgano. El compuesto se aplicó en el intervalo de concentraciones de 10⁻⁸ mol/l a 10⁻⁵ mol/l (Figura 3, Tabla 1). El vardenafilo relajó los anillos uretrales con un valor de CE₅₀ de 0,96 µmol/l y las tiras de próstata y de vejiga con el valor de CE50 de 1,1 y 5,0 µmol/l, respectivamente.

Concentración (µmol/l)	Vejiga (%) contracción)	Uretra (%) contracción)	Próstata (%) contracción)
0,001	94,3 ± 0,9	88,4 ± 2,4	-
0,01	91,2 ± 1,3	88,5 ± 2,1	-
0,1	89,9 ± 1,8	77,1 ± 3,9	99,2 ± 3,0
1	77,8 ± 2,1	44,9 ± 1,5	76,0 ± 2,3
10	25,3 ± 3,2	3,5 ± 1,7	25,3 ± 3,2

Tabla 1: Efectos del vardenafilo sobre la contracción de tejidos urogenitales de rata aislados. La relajación se expresa en forma de porcentaje de la pre-contracción. Cada punto representa un valor medio $\pm$ ETM, n= 9.

Ejemplo 3

- 5 Preparación de tejido: Se procedió a la eutanasia de ratas Wistar macho (200-300 g) usando dióxido de carbono. Los tejidos se extrajeron y se colocaron en tampón de Krebs-Henseleit enfriado con hielo de la siguiente composición (en mmol/l): NaCl 112, KCl 5,9, CaCl₂ 2,0, MgCl₂ 1,2, NaH₂PO₄ 1,2, NaHCO₃ 25, glucosa 11,5. Del cuerpo de la vejiga se cortaron cuatro tiras longitudinales de
- 10 igual tamaño de aproximadamente 2 mm x 10 mm. Tiras de próstata se obtuvieron cortando transversalmente a través de los lóbulos de la glándula prostática en paralelo a la uretra. De la parte proximal de la uretra se diseccionó un anillo por animal.

- Conejos blancos New Zealand se anestesiaron usando tiopental. Se
- 15 extrajo la vejiga urinaria y se colocó en tampón de Krebs-Henseleit enfriado con hielo de la siguiente composición (en mmol/l): NaCl 112, KCl 5,9, CaCl₂ 2,0, MgCl₂ 1,2, NaH₂PO₄ 1,2, NaHCO₃ 25 y glucosa 11,5. Del cuerpo de la vejiga se cortaron tiras longitudinales de igual tamaño de 2 mm x 10 mm.

- Registro de la actividad mecánica: Las preparaciones se transfirieron a
- 20 baños del órgano de 20 ml que contienen solución de Krebs-Henseleit equilibrada con O₂ al 95%, CO₂ al 5% a 37°C. Las tiras se montaron entre dos ganchos por medio de dos clip. Para registrar la tensión isométrica, uno de los ganchos se conectó a un transductor de fuerza que a su vez estaba unido a un amplificador y registrador de gráficos. El otro gancho estaba unido a una
- 25 unidad móvil, lo que permite un ajuste preciso de la tensión de precarga. A

continuación se dio a todos los tejidos un periodo de equilibrado de 60 min, durante los cuales se lavaron y la tensión de reposo se ajustó a 1 g cada 20 min.

Tras el periodo de equilibrado, cada experimento se inició exponiendo la
5 preparación a solución de Krebs-Henseleit K⁺ (50 mmol/l). El procedimiento se repitió 3 veces y los tejidos se lavaron al menos tres veces entre cada contracción.

A continuación, las tiras de vejiga se contrajeron previamente usando
solución de Krebs-Henseleit K⁺ (50 mmol/l). Cuando la contracción se
10 estabilizó se construyó una curva de respuesta a la dosis acumulativa del compuesto analizado. La contracción estabilizada inducida por la solución de Krebs-Henseleit K⁺ (50 mmol/l) se definió como una tensión del 100%. La relajación se expresó en forma de tensión en porcentaje.

Tiras prostáticas y anillos uretrales se contrajeron previamente usando
15 10⁻⁶ mol/l de fenilefrina. Los efectos de los compuestos sobre el tejido prostático se analizaron de un modo no acumulativo con etapas de lavado entre cada concentración.

Efectos de los inhibidores de la PDE5 y la PDE4 sobre tiras de vejiga de conejo aisladas: Los efectos del inhibidor de la PDE5 vardenafilo y del inhibidor
20 de la PDE4 Roflumilast sobre la relajación del músculo liso de la vejiga se analizaron en el baño de órgano usando tiras de vejiga de conejo. Ambos compuestos se analizaron en la concentración de 10⁻⁹ mol/l a 10⁻⁵ mol/l (Fig. 4, Tab. 2). Roflumilast y vardenafilo relajaron las tiras de vejiga con una CI₅₀ de 260 nmol/l y 1,7 µmol/l respectivamente (Tabla 2).

Concentración (μmol/l)	Roflumilast (%) contracción)	Vardenafilo (%) contracción)
0,001	100,8 ± 0,6	100,9 ± 1,0
0,01	90,1 ± 1,7	99,9 ± 0,9
0,1	75,1 ± 2,1	95,7 ± 1,7
1	55,8 ± 2,5	76,3 ± 3,7
10	40,9 ± 3,3	9,6 ± 12,6

Tabla 2: Efectos de roflumilast y vardenafilo sobre la contracción de tiras de vejiga de conejo aisladas. La relajación se expresa en forma de porcentaje de la pre-contracción. Cada punto representa un valor medio ± ETM, n= 9.

Ejemplo 4

Todos los experimentos con animales se realizaron según la “Ley alemana para la protección de los animales de laboratorio” y se llevaron a cabo según las normas aprobadas del permiso “Tierversuchsvorhaben N° 401/A01 M010/M011 vom 09.07.2004”. Los experimentos se realizaron con ratas Sprague Dawley hembra con un peso corporal entre 200-250 g.

Obstrucción del tracto de salida de la vejiga: Para la obstrucción del tracto de salida de la vejiga se anestesió a las ratas con una mezcla de isoflurano 1,5-2% en un portador de N₂O al 66%, O₂ al 33%. El abdomen se afeitó, se abrió mediante una incisión en la línea media inferior, se identificaron la vejiga y la uretra, y se expuso la unión uretravesical. A lo largo de la uretra proximal se colocó una barra de metal de 1,0 mm y se ató fuertemente una ligadura de nylon 6-0 alrededor de la uretra y la barra. A continuación se extrajo

la barra y el abdomen se cerró mediante una ligadura de seda y se limpió con etanol al 70%. Se dispuso de un tratamiento postoperatorio anti-dolor con Rimadyl® 10 mg/kg (Pfizer). Después, las ratas se conservaron durante 2 ETManas y se alimentaron con agua corriente y pienso estándar para ratas. 24
5 horas antes de la cistometría, las ratas se anestesiaron con isoflurano tal y como se ha descrito antes. La laparotomía se realizó como se ha descrito antes, se expuso la vejiga y se implantó un catéter de polietileno (PE50) en la bóveda de la vejiga. El catéter se tuneló subcutáneamente usando una cánula para alcanzar la parte posterior del cuello del animal. Además, en la vena
10 yugular se colocó un catéter para administración intravenosa (PE10) y se tuneló subcutáneamente hasta la parte posterior del cuello del animal. Ambos catéteres se fijaron mediante una sutura y esparadrapo.

Cistometría consciente: Para la cistometría los animales se anestesiaron brevemente mediante isoflurano como se ha descrito antes, se colocaron en
15 una jaula Ballman y se fijaron. A continuación, los animales fueron recuperados al menos durante 1 h antes del inicio del experimento. Después, el catéter de la vejiga se conectó al tubo de forma T para conectar un transductor de presión para la medición de la presión intravesical (MLT0698, ADInstruments) y una bomba de infusión (Percusor Compact®, Braun Melsungen) para la infusión
20 continua de solución salina a un caudal de 10 ml/h. Los animales con OSV mostraron un incremento en la capacidad de la vejiga (debido al aumento de tamaño de la vejiga) y de las contracciones de no vaciado (que simula los síntomas irritativos de la HPB), cuando se compararon con un animal control. La eficacia del tratamiento se cuantificó mediante cálculo de las contracciones
25 de no vaciado por intervalo de micción antes y después del tratamiento. Para el

control positivo se usó el antagonista de receptores alfa tamsulosina (10 µg/kg). Los valores se dieron en % de reducción de las contracciones de no vaciado.

Análisis estadístico de los resultados: Los datos se expresan en media ± error típico de la media (ETM) y *n* indica el número de experimentos. La significación de las diferencias entre medias se determinó mediante la prueba t de Student pareada y no pareada. Los niveles de probabilidad inferiores a 0,05 se consideraron significativos.

Efecto del vardenafilo sobre las contracciones de no vaciado en ratas con OSV: Para el modelo de OSV se realizó una ligadura parcial de la uretra en las ratas con anestesia. La obstrucción del tracto de salida de la vejiga (OSV) resultante de este procedimiento causó un incremento significativo del peso de la vejiga (datos no mostrados), lo que indica una pronunciada hipertrofia de la vejiga. También produjo contracciones de no vaciado (CNV) de la vejiga, que se detectaron mediante cistometría en animales conscientes. Estas CNV fueron una medida de los síntomas irritativos en la HPB y se redujeron de forma significativa con un MED de 3 mg/kg de vardenafilo i.v.

Ejemplo 5

Todos los experimentos con animales se realizaron según la "Ley alemana para la protección de los animales de laboratorio" y se llevaron a cabo según las normas aprobadas del permiso "Tierversuchsvorhaben N° 401/A01 M010/M011 vom 09.07.2004". Los experimentos se realizaron con ratas Sprague Dawley hembra con un peso corporal entre 200-250 g.

Cistometría con anestesia: Para la cistometría se anestesió a ratas SD hembra con uretano (1,2 g/kg, ip). Tras la laparotomía, se expuso la vejiga se y ambos uréteres se ligaron y cortaron. En la bóveda de la vejiga se implantó una cánula

de polietileno (PE50) y se cerró el abdomen. El catéter de la vejiga se conectó al tubo con forma de T para conectar una bomba de infusión (Perfusor® compact; Braun Melsungen) para la infusión continua de solución salina y para conectar un transductor de presión (Combitrans; Braun Melsungen) para la medición de la presión intravesical. Las señales de presión intravesical se registraron con el Powerlab system (MLT0698, ADInstrument). La cistometría se realizó tras un periodo de equilibrado de 1 h desde el procedimiento quirúrgico. Para el tratamiento farmacológico i.v., la vena femoral izquierda se canuló con un catéter de polietileno. El efecto de los tratamientos se calculó en el intervalo de micción (correspondiente a la capacidad de la vejiga).

Se realizó la inducción de una vejiga hiperactiva con una infusión en la vejiga de solución al 0,2% de ácido acético (diluido con solución salina) en lugar de solución salina o con inyección i.p. de 150 mg/kg de ciclofosfamida 18 h antes de la cistometría.

15 Análisis estadístico de los resultados: Los datos se expresan como la media $\pm$ error típico de la media (ETM) y n indica el número de experimentos. La significación de las diferencias entre medias se determinó mediante la prueba t de Student pareada y no pareada. Los niveles de probabilidad inferiores a 0,05 se consideraron significativos.

20 Efecto del vardenafilo sobre el intervalo de micción en ratas tratadas con CYP: El intervalo de micción aumentó de forma significativa con un MED de 3 mg/kg de vardenafilo i.v.

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Habiendo descrito la invención como antecede, se declara como propiedad lo contenido en las siguientes

REIVINDICACIONES

- 5 1. Un procedimiento de detección selectiva para los inhibidores de la
PDE-5 útiles como agentes terapéuticos en el tratamiento de una
enfermedad comprendida en un grupo de enfermedades constituidas
por hiperplasia prostática benigna (HPB), obstrucción del tracto de
salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI),
10 que comprende las etapas de i) poner en contacto un compuesto de
prueba con un polipéptido de PDE-5, ii) determinar la actividad del
polipéptido de PDE5 a cierta concentración del compuesto de prueba
o en ausencia de dicho compuesto de prueba, iii) determinar la
actividad de dicho polipéptido de PDE5 a una concentración diferente
15 de dicho compuesto de prueba, iv) seleccionar al menos un
compuesto con efecto inhibidor sobre el polipéptido de PDE-5.

- 20 2. Un procedimiento de detección selectiva para los inhibidores de la
PDE-4 útiles como agentes terapéuticos en el tratamiento de una
enfermedad comprendida en un grupo de enfermedades constituidas
por hiperplasia prostática benigna (HPB), obstrucción del tracto de
salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI),
que comprende las etapas de i) poner en contacto un compuesto de
prueba con un polipéptido de PDE-4, ii) determinar la actividad del
25 polipéptido de PDE4 a cierta concentración del compuesto de prueba

o en ausencia de dicho compuesto de prueba, iii) determinar la actividad de dicho polipéptido de PDE4 a una concentración diferente de dicho compuesto de prueba, iv) seleccionar al menos un compuesto con efecto inhibidor sobre el polipéptido de PDE-4.

- 5
3. Un procedimiento de detección selectiva para combinaciones de inhibidores de la PDE5 e inhibidores de la PDE4 útiles como agentes terapéuticos en el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática
- 10 benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), que comprende las etapas de i) poner en contacto un primer compuesto de prueba con un polipéptido de PDE-5, ii) determinar la actividad del polipéptido de PDE5 a una cierta concentración del primer compuesto de prueba o
- 15 en ausencia de dicho primer compuesto de prueba, iii) determinar la actividad del polipéptido de PDE5 a una concentración diferente de dicho primer compuesto de prueba, iv) seleccionar al menos un primer compuesto con efecto inhibidor sobre el polipéptido de PDE-5, v) poner en contacto un segundo compuesto de prueba con un
- 20 polipéptido de PDE4, vi) determinar la actividad del polipéptido de PDE4 a cierta concentración del segundo compuesto de prueba o en ausencia de dicho segundo compuesto de prueba, vii) determinar la actividad del polipéptido de PDE4 a diferente concentración de dicho segundo compuesto de prueba, vii) seleccionar al menos un segundo
- 25 compuesto con efecto inhibidor sobre el polipéptido de PDE4, vii)

combinar al menos un compuesto con actividad inhibidora de PDE5 con al menos un compuesto que posea actividad inhibidora de PDE4.

- 5 4. Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), trastornos genitourinarios comprendidos por síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como 10 incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico 15 como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas y disfunción eréctil en un mamífero, que comprende un agente terapéutico que regula la actividad de un polipéptido de PDE5.
- 20 5. Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) 25 en un mamífero, que comprende un agente terapéutico que regula la

actividad de un polipéptido de PDE4.

- 5 6. Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero, que comprende un agente terapéutico que es una combinación de los agentes terapéuticos de las reivindicaciones 4 y 5.
- 10 7. Una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI),
- 15 trastornos genitourinarios que comprenden síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y
- 20 mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas y disfunción eréctil en un mamífero, que
- 25 comprende un inhibidor de PDE-5 seleccionado del grupo de

inhibidores de PDE-5 constituidos por vardenafilo, sildenafil, tadalafilo, udenafilo, dasantafilo, avanafilo, SLx2101 y LAS34179.

5 8. Uso de un inhibidor de PDE5 para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), trastornos genitourinarios que comprenden síndrome vesical neurogénico (VHA) y (CI),
10 incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales
15 mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas y disfunción eréctil en un mamífero.

20 9. Uso de un inhibidor de PDE4 para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero.

25

10. Uso de una combinación de al menos un inhibidor de PDE4 y al menos un inhibidor de PDE5 para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero.
- 5
11. Uso de un agente que es un inhibidor de un polipéptido de PDE4 y un polipéptido de PDE5 para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero.
- 10
12. Uso de un inhibidor de PDE-5 seleccionado del grupo de inhibidores de PDE-5 constituido por vardenafilo, sildenafil, tadalafilo, udenafilo, dasantafilo, avanafilo, SLx2101 y LAS34179 para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), trastornos genitourinarios que comprenden síndrome vesical neurogénico (VHA) y (CI), incontinencia urinaria (IU), como incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM, IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los
- 15
- 20
- 25

órganos que constituyen el sistema genitourinario de varones y mujeres, enfermedades renales como insuficiencia renal crónica o aguda, enfermedades renales mediadas por el sistema inmunológico como rechazo de trasplante renal, nefritis lúpica, enfermedades renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía tóxica, uropatías obstructivas y disfunción eréctil en un mamífero.

13. Uso de una combinación de al menos un inhibidor de PDE-4 seleccionado del grupo de inhibidores de PDE-4 constituido por roflumilast, cilomilast y Piclamilast y al menos un inhibidor de PDE-5 seleccionado del grupo de inhibidores de PDE-5 constituido por vardenafilo, sildenafil y tadalafilo para la preparación de una composición farmacéutica para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI) en un mamífero.

14. Una composición farmacéutica que contiene al menos un compuesto seleccionado del grupo vardenafilo, sildenafil, tadalafilo, udenafilo, dasantafilo, avanafilo, SLx2101 y LAS34179 o una sal, un hidrato o un hidrato de una sal de los mismos, para el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV), síntomas del tracto urinario inferior (STUI),

trastornos genitourinarios que comprenden síndrome vesical
neurogénico (VHA) y (CI), incontinencia urinaria (IU), como
incontinencia mixta, de urgencia, de tensión o por sobreflujo (IUM,
IUU, IUE, IUS), dolor pélvico, trastornos benignos y malignos de los
5 órganos que constituyen el sistema genitourinario de varones y
mujeres, enfermedades renales como insuficiencia renal crónica o
aguda, enfermedades renales mediadas por el sistema inmunológico
como rechazo de trasplante renal, nefritis lúpica, enfermedades
renales por complejo inmunitario, glomerulopatías, nefritis, nefropatía
10 tóxica y uropatías obstructivas en un mamífero.

15. Una composición farmacéutica que contiene al menos un compuesto
seleccionado del grupo vardenafilo, sildenafil, tadalafilo, udenafilo,
dasantafilo, avanafilo, SLx2101 y LAS34179 o una sal, un hidrato o
15 un hidrato de una sal de los mismos, para el tratamiento de la vejiga
neurogénica, la vejiga hiperactiva y la cistitis intersticial.

16. Una composición farmacéutica que contiene vardenafilo o una sal, un
hidrato o un hidrato de una sal del mismo, para el tratamiento de la
20 vejiga neurogénica, la vejiga hiperactiva y la cistitis intersticial.

17. Uso de al menos un compuesto seleccionado del grupo vardenafilo,
sildenafil, tadalafilo, udenafilo, dasantafilo, avanafilo, SLx2101 y
Las34179 o una sal, un hidrato o un hidrato de una sal de los
25 mismos, para la preparación de una composición farmacéutica para

el tratamiento de la vejiga neurogénica, la vejiga hiperactiva y la cistitis intersticial.

18. 5 Uso de vardenafilo o una sal, un hidrato o un hidrato de una sal del mismo, para la preparación de una composición farmacéutica para el tratamiento de la vejiga neurogénica, la vejiga hiperactiva y la cistitis intersticial.

Fig. 2

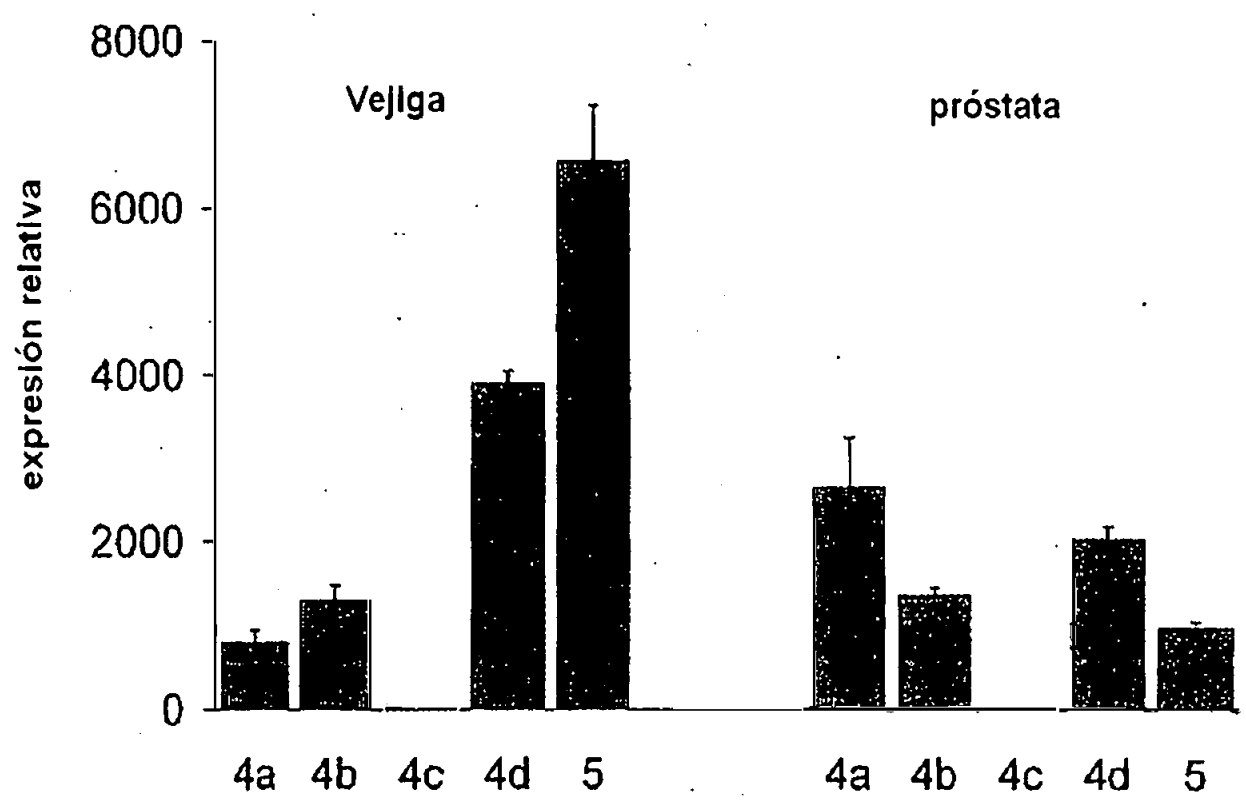


Fig. 3

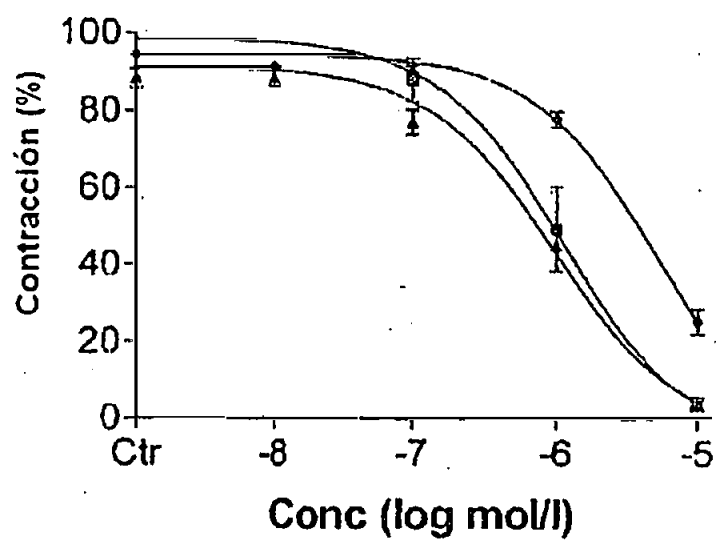
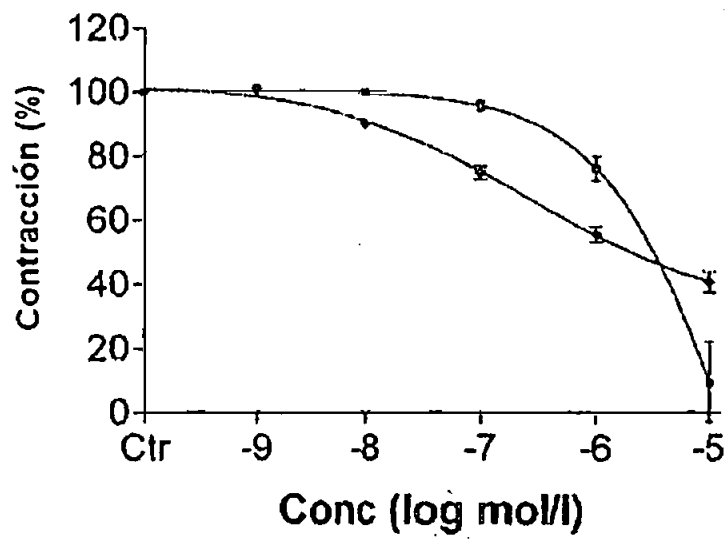


Fig. 4



5/6

Fig.5

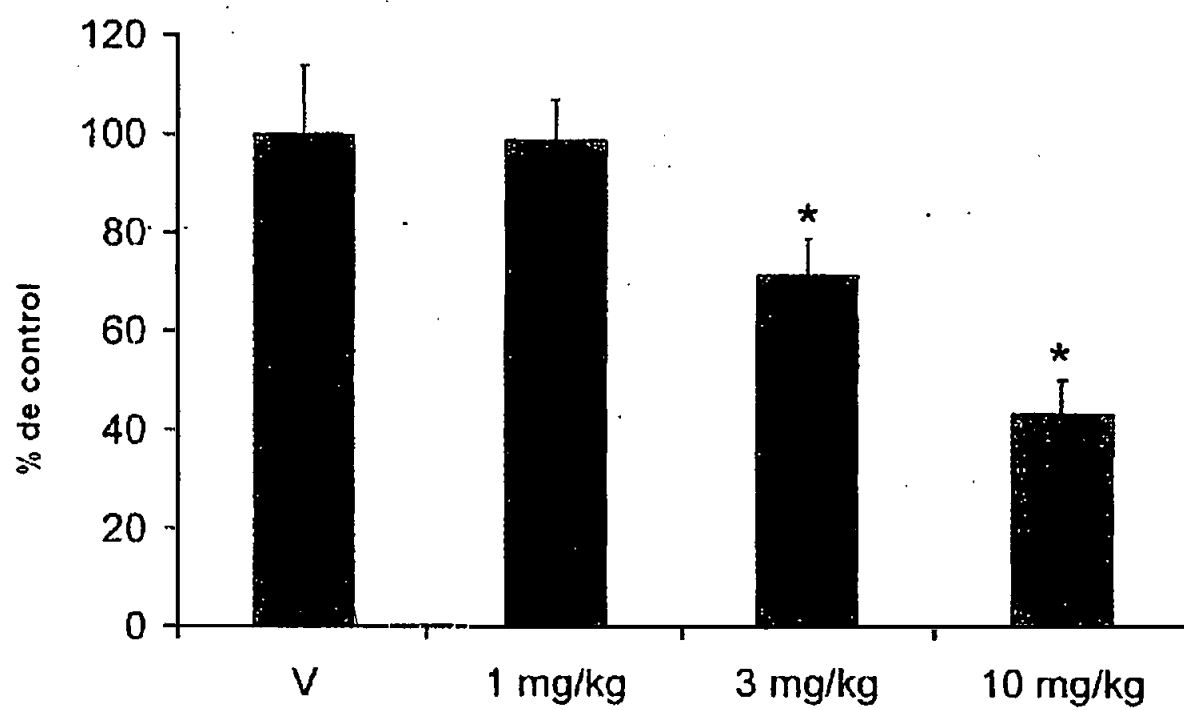
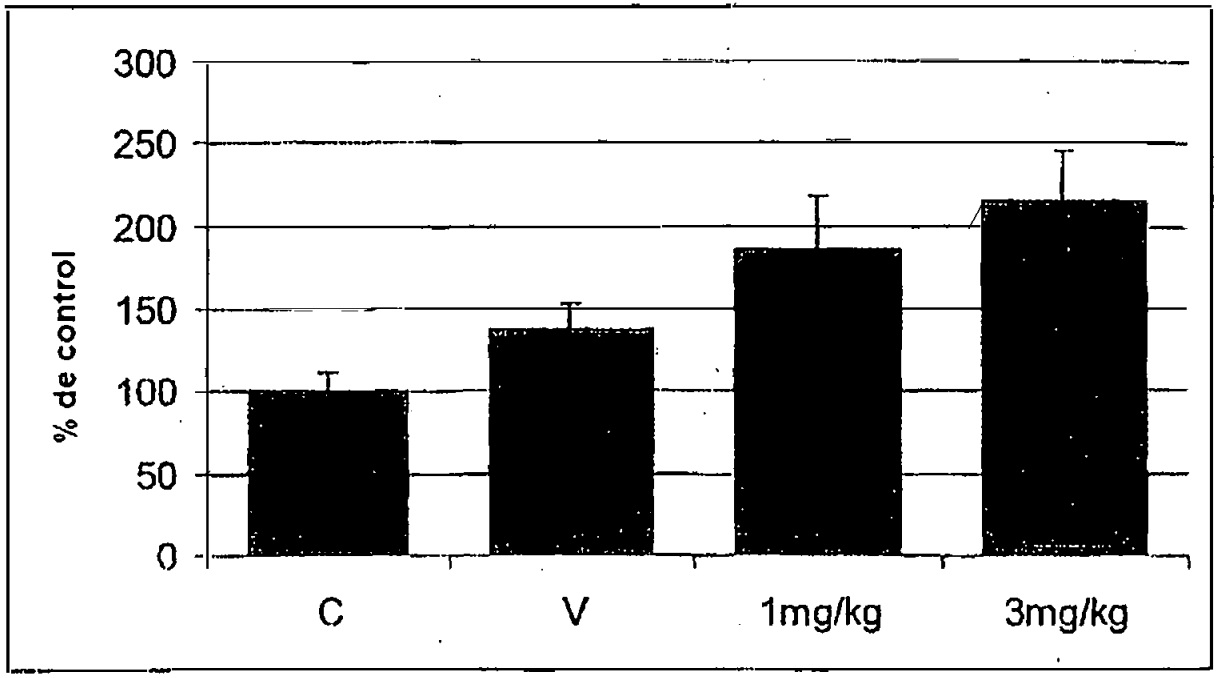


Fig. 6



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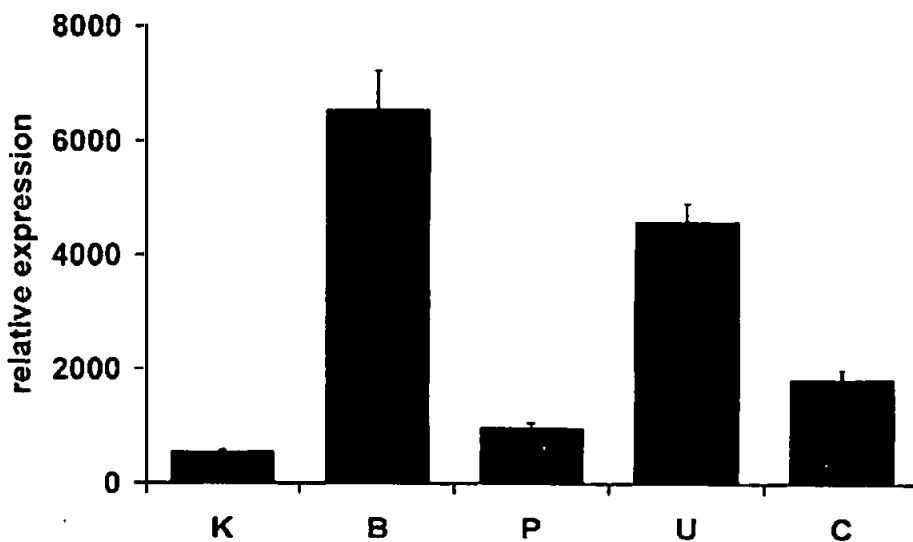
- (51) International Patent Classification:
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05021261.2 29 September 2005 (29.09.2005) EP
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- (71) Applicant (for all designated States except US): BAYER
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- (72) Inventors; and
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- (74) Common Representative: BAYER HEALTHCARE
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Leverkusen (DE).
- (81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP,
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LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ,
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kind of regional protection available): ARIPO (BW, GH,
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GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

[Continued on next page]

(54) Title: PDE INHIBITORS AND COMBINATIONS THEREOF FOR THE TREATMENT OF UROLOGICAL DISORDERS



(57) Abstract: The invention provides pharmacological compositions comprising PDE-5 and PDE-4 inhibitors, alone or in combination, for the treatment of urological disorders comprising Benign Prostate Hyperplasia (BPH), Lower Urinary Tract Symptoms (LUTS) and in particular irritative symptoms caused by BPH-induced bladder outlet obstruction (BOO). The invention also provides methods of screening for such PDE-5 and PDE-4 inhibitors for use, alone and in combination, in the preparation of medicaments for the treatment of said urological disorders.

WO 2007/039075 A3



— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(88) Date of publication of the International search report:
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/009040

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/44 A61P13/00 A61K31/00

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)
C12Q

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, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2005/105073 A (MEDICINOVA INC [US]; SMITH CHARLES [US]; LOCKE KENNETH WALTER [US]; KI) 10 November 2005 (2005-11-10) claim 1; figure 5	2
X	WO 01/17480 A (ANDROSOLUTIONS INC [US]) 15 March 2001 (2001-03-15) claim 2	2
X	DE 195 40 642 A1 (STIEF CHRISTIAN GEORG PRIV DOZ [DE]) 7 May 1997 (1997-05-07) claims 1-5	1,3,4, 6-8,10, 11

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

☒ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

28 March 2007

Date of mailing of the international search report

07/05/2007

Name and mailing address of the ISA/

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Rosin, Oliver

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/009040

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	UCKERT ET AL: "CHARACTERIZATION AND FUNCTIONAL RELEVANCE OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ISOENZYMES OF THE HUMAN PROSTATE" JOURNAL OF UROLOGY, BALTIMORE, MD, US, vol. 166, no. 6, December 2001 (2001-12), pages 2484-2490, XP005543479 ISSN: 0022-5347 cited in the application abstract; figure 3; table 4	1,3,4, 6-8, 10-12, 14,15,17
X	MONTORSI F ET AL: "REVIEW OF PHOSPHODIESTERASES IN THE UROGENITAL SYSTEM: NEW DIRECTIONS FOR THERAPEUTIC INTERVENTION" JOURNAL OF SEXUAL MEDICINE, BLACKWELL PUBLISHING LTD., OXFORD, GB, vol. 1, no. 3, 2004, pages 322-336, XP009077256 ISSN: 1743-6095 abstract; table 2 page 326	4,6-8, 10-12, 14-18
X	LIN C-S ET AL: "PHOSPHODIESTERASES AS THERAPEUTIC TARGETS" UROLOGY, BELLE MEAD, NJ, US, vol. 61, no. 4, April 2003 (2003-04), pages 685-691, XP001181223 ISSN: 0090-4295 page 689	4,6-8, 10-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/009040

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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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:

see additional sheet

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.:
1, 3-4, 7-8, 10-18 (all fully) and 6 (in part)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 3-4, 7-8, 10-18 (all fully) and 6 (in part)

Methods, compositions and uses of PDE5 inhibitors in bladder disease.

2. claims: 2-3, 5, 9-11, 13 (all fully) and 6 (in part)

Methods, compositions and uses of PDE4 inhibitors in bladder disease.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2006/009040

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005105073 A	10-11-2005	AU 2005237607 A1	10-11-2005
		CA 2564731 A1	10-11-2005
		EP 1744740 A1	24-01-2007
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		EP 1214039 A2	19-06-2002
		WO 0117479 A2	15-03-2001
DE 19540642 A1	07-05-1997	NONE	

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Bayer HealthCare

Telefax No.: Fax-No: 0 0041-22338 8270 No. of Page(s): 1

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12. März 2008

PCT/EP2006/009040
Bayer HealthCare AG et al.

BHC 05 1111-WO

KK/Lin/et

Request for correction according to rule 91

The nationality of the inventor/applicant TINEL, Hanna is

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Law and Patents
Patents and Licensing
51368 Leverkusen
Germany

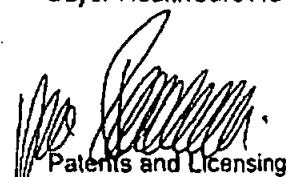
BE/PL

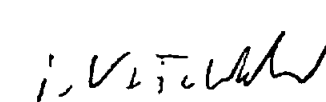
Tel. +49 (0)214-30-7 11 66
Fax +49 (0)214-30-96-7 77 17
BBS-PatentService@bayerbbs.com
www.BayerBBS.com

It is hereby requested to make the correction ex officio.

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HRB 49894

 Industria y Comercio SUPERINTENDENCIA		SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO DIVISION DE NUEVAS CREACIONES EXTRACTO PARA PUBLICACIÓN SOLICITUD DE PATENTE DE INVENCION PCT	
(21) N° de solicitud:		(51) Int Cl:	
22) Fecha de solicitud:		(71) Solicitante(s) BAYER HEALTHCARE AG	
(30) Prioridad	(31) No. Prioridad	(32) Fecha	(33) Pais
	05021261.2	29-09-05	EP
	08007776.5	13-04-06	EP
		(72) Inventor(es) ULBRICH, Ernst; SANDNER, Peter; TINEL, Hanna HÜTTER, Joachim	
		(74) Apoderado: JAIME EDUARDO DELGADO VILLEGAS	
(85) Fecha limite inicio fase nacional:		29-04-2008	
(86) Datos relativos a la presentación de la solicitud PCT		(87) Publicación Internacional	
Fecha de presentación de la solicitud:		Fecha: 12-04 - 07 Fecha (dd/mm/aa)	
No. de solicitud PCT/EP2006/009040		N° publicación: WO2007/039075 A2	
(54) Título: INHIBIDORES DE LA PDE Y COMBINACIONES DE LOS MISMOS PARA EL TRATAMIENTO DE TRASTORNOS UROLÓGICOS			

Resumen - Reivindicación(es):

Un procedimiento de detección selectiva para los inhibidores de la PDE-5 útiles como agentes terapéuticos en el tratamiento de una enfermedad comprendida en un grupo de enfermedades constituidas por hiperplasia prostática benigna (HPB), obstrucción del tracto de salida de la vejiga (OSV) y síntomas del tracto urinario inferior (STUI), que comprende las etapas de i) poner en contacto un compuesto de prueba con un polipéptido de PDE-5, ii) determinar la actividad del polipéptido de PDE5 a cierta concentración del compuesto de prueba o en ausencia de dicho compuesto de prueba, iii) determinar la actividad de dicho polipéptido de PDE5 a una concentración diferente de dicho compuesto de prueba, iv) seleccionar al menos un compuesto con efecto inhibidor sobre el polipéptido de PDE-5.

ESTA CERTIFICACION DE EXCEDENTE DE PAGO SOLU PODRA SER UTILIZADA
COMO PARTE DE LA CANCELACION DE UNA TARIFA VIGENTE Y NO PODRA SER
SUSTITUIDA POR NINGUN RECIBO.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Fecha: 2008-03-28 13:47:17 Dep. 2020 NUECREACION
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Act. 411 PRESENTACION Folios: 140

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

MIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 25,476
FECHA : MARZO 28 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
MARIO DELGADO ECHEVERRY	RECIBO DE CAJA			25473	28/03/2008	103,000.00
MARIO DELGADO ECHEVERRY	RECIBO DE CAJA			25474	28/03/2008	401,600.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		1 TRAMITES DE SOL. DE PATENTE DE IN	501,000.00
		TOTAL :	\$ 501,000.00

SON: QUINIENTOS UN MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

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☒

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COMPLETA ☐

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28 MAR. 2008

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142

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
DELGADO VILLEGAS JAIME EDUARDO

REFERENCIA	Radicación No.	8 30778
	Solicitud internacional	PCT/EP2006/009040
	Fecha inicio fase nacional	29/04/2008
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	6746

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

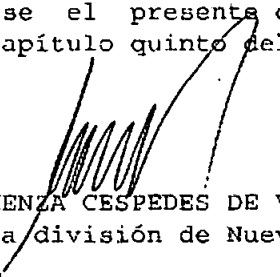
1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.

2. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

3. Se indica al solicitante que no se tendrá en cuenta la información presentada en el documento fase nacional (folios 9 a 37), por no corresponder a la información relativa a la publicada en fase internacional.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No.10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 29 MAYO 2008
adpali