(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 November 2007 (01.11.2007)

PCT

(10) International Publication Number
WO 2007/121963 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01) *A61K 31/165* (2006.01)

(21) International Application Number:
PCT/EP2007/003535

(22) International Filing Date: 23 April 2007 (23.04.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
06008620.4 25 April 2006 (25.04.2006) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, 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, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

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.

WO 2007/121963 A1

(54) **Title:** METHODS FOR INTERFERING WITH DISEASE RELATED TO IMPAIRED MAST CELL ACTIVATION

(57) **Abstract:** A method for interfering with disorders that depend on mast cell activation comprising, contacting mast cells expressing serum and glucocorticoid inducible kinase (SGK) with a substance that modulate glucocorticoid inducible kinase and thereby modulate the PI3 kinase dependent ion channel regulation and function. Furthermore the invention relates to methods for the diagnosis and for the identification of compounds that are useful for the detection or therapy of inflammatory disease.

Methods for interfering with disease related to impaired mast cell activation

Background of the invention

Mast cells express a large number of IGE receptors (FcεRI) and largely account for the IgE dependent allergic reactions [Kawakami and Galli 2002; Kawakami and Kitaura 2005], such as allergic rhinitis [Pawankar 2005], asthma [Bradding 2003], anaphylactic and delayed hypersensitivity [Askenase et al., 1983; Conti et al., 1992; Galli et al., 2005; Van Loveren et al., 1983]. They are considered to critically participate in the pathophysiology of diverse inflammatory diseases including psoriasis [Cao et al., 2005; Jamieson et al., 2005; Kanda and Watanabe 2003; Kawaguchi et al., 2005; Shepherd et al., 2004; Theoharides et al., 2004], atopic dermatitis [Theoharides et al., 2004], rheumatoid arthritis [Jonsson et al., 2005; Juurikivi et al., 2005], Crohn´s disease [Gebhardt et al., 2005], irritable bowel syndrome [Gebhardt et al., 2005] and male infertility [Agarwal et al., 1987; Dupont et al., 2000; Frungieri et al., 2002; Nagai et al., 1992; Yamanaka et al., 2000]. Mast cells release a variety of cytokines and thus regulate the function of other inflammatory cells such as neutrophils and T cells [Asada et al., 1997; Biedermann et al., 2000; Galli et al., 2005; Galli and Nakae 2003; Hill et al., 1996; Nakae et al., 2005; Nakae et al., 2006]. Mast cell function is under tight regulation. Activation of the mast cells involves the stimulation of the PI3 kinase pathway [Andrade et al., 2004; Gilfillan and Tkaczyk 2006; Wymann et al., 2003] and is suppressed by glucocorticoids [Andrade et al., 2004; Collado-Escobar et al., 1990; Fushimi et al., 1998; Krishnaswamy et al., 1997; Matsuda et al., 2005; Mazingue et al., 1978; Robin et al., 1985; Wershil et al., 1995]. Activation of mast cells further involves activation of Ca^{2+} channels [Bradding et al., 2003; Buess et al., 1999; Dernick et al., 2003; Duffy et al., 2001a; Duffy et al., 2001b; Kahr et al., 2004; Mazurek et al., 1980; Stokes et al., 2004], K^+ channels [Bradding et al., 2003;

Bradding 2005; Duffy et al., 2005; Mark et al., 2004] and Cl^- channels [Duffy et al., 2001a; Duffy et al., 2001b].

Signalling molecules downstream of PI3kinase include the serum and glucocorticoid inducible kinase SGK1 [Lang and Cohen 2001], which is expressed in all tissues tested [Waldegger et al., 1997]. SGK1 has been shown to regulate a wide variety of ion channels [Lang et al., 2003]. The kinase contributes to the regulation of salt appetite [Vallon et al., 2005], renal electrolyte excretion [Huang et al., 2004; Wulff et al., 2002] and blood pressure [Huang et al., 2006]. It participates in the regulation of intestinal transport [Grahammer et al., 2006] and peripheral glucose uptake [Palmada et al., 2006]. Several observations point to its pivotal role in fibrosing disease [Feng et al., 2005; Lang et al., 2000; Vallon et al., 2006; Waldegger et al., 1999]. Even though SGK1 is a glucocorticoid sensitive gene [Firestone et al., 2003], nothing is known about its putative role in inflammatory disease. Most recent observations disclosed its role in the regulation of insulin release from pancreatic beta cells [Ullrich et al., 2005].

The present study has thus been performed to elucidate whether PI3 kinase dependent ion channel regulation and function of mast cells similarly involves SGK1. Thus, the function of mast cells has been studied in gene targeted mice lacking SGK1 (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$).

Summary of the invention

The current invention elucidates the role of SGK1 in mast cell function. It is shown for the first time that SGK1 is involved in the regulation of mast cells by the PI3 kinase pathway and plays a pivotal role in the stimulation of mast cells.

Kinases activated through the PI3 kinase pathway include the serum- and glucocorticoid-inducible kinase 1 (SGK1).

Mast cells isolated and cultured from the bone marrow (BMCMC) of SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$) have been evaluated with respect to their cytosolic Ca^{2+} activity (determined utilizing Fura2 fluorescence), their channel activity by patch clamp technique and furthermore measured by FACS analysis. As a result, forward and side FACS scatter were both significantly smaller in *sgk1*$^{-/-}$ BMCMC than in *sgk1*$^{+/+}$ BMCMC, and this points to a decrease of cell volume and granulation. Moreover, Ca^{2+} entry following Ca^{2+} depletion was significantly reduced in *sgk1*$^{-/-}$ BMCMC when compared to *sgk1*$^{+/+}$ BMCMC and this points to a defective activation of the Ca^{2+} release activated Ca^{2+} channel I_{CRAC}. Accordingly, Ca^{2+} sensitive K^+ channels were activated by IgE-DNP in *sgk1*$^{+/+}$ BMCMC but not in *sgk1*$^{-/-}$ BMCMC. Treatment of the cells with the Ca^{2+} ionophore ionomycin (1 µM) led to similar activation of the K^+ channels in both genotypes, indicating that the Ca^{2+} sensitive K^+ channels are similarly expressed and similarly sensitive to activation by Ca^{2+} in *sgk1*$^{+/+}$ BMCMC and *sgk1*$^{-/-}$ BMCMC. Thus, the defective regulation of K^+ channels unexpectedly results from blunted increase of cytosolic Ca^{2+} activity. Further studies have been performed to explore whether the impaired stimulation of Ca^{2+} entry in *sgk1*$^{-/-}$ BMCMC affects the *in vivo* function of mast cells. To this end, Trinitrochlorobenzene-induced contact hypersensitivity reaction has been tested in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice. As a result, the early ear swelling following stimulation with Trinitrochlorobenzene (TNCB) was completely lacking in *sgk1*$^{-/-}$ mice, an

observation indeed pointing to severe impairment of mast cell function *in vivo*. The observations unravel for the first time a critical role of SGK1 in the ion channel regulation and function of mast cells, and thus disclose a novel molecular functional switch in the regulation of allergic reaction.

The current unexpected findings make serum and glucocorticoid inducible kinase-1, modulators of SGK-1 activity and especially antagonists of SGK-1 activity important for the therapy of allergic reaction and other diseases driven by mast cell activity.

Based on the new and unexpected function of SGK1 in mast cell activation the current invention delivers a method for inhibiting activation of mast cells by contacting said mast cells with substances that inhibit glucocorticoid inducible kinase and thereby modulate the PI3 kinase dependent ion channel regulation and function.

An especially useful molecular target for inhibiting activation of mast cells according to this invention are glucocorticoid inducible kinases selected from the group consisting of: SGK1, SGK2, SGK3.

Moreover a method for treating mast cell activation dependent disorders is readily envisible and this does require to administering substances that inhibit SGK1 to patients in need of such a treatment. The preparation of a medicament on the basis of an SGK1 inhibitor is generally known in the art.

Preferred disorders that involve mast cell activation are but are not limited to allergic reaction, allergic rhinitis, anaphylactic and delayed hypersensitivity, psoriasis, atopic dermatitis, rheumatoid arthritis, Crohn´s disease, irritable bowel syndrome or male infertility.

The listed disorders would greatly benefit from a treatment with SGK1 inhibiting or modulating compounds. Modulating SGK1 compounds may be prefered in instances where the complete inhibition of SGK1 is not required and controlled residual SGK1 activity is needed. Use of SGK inhibitors selected from the listed compounds having the general formula I or II for the manufacture of a medicament for the treatment of disorders caused by mast cell activation. A list of SGK inhibitors and modulators having the general

formula I or II for the manufacture of a medicament for the treatment of disorders caused by mast cell activation.

The treatment of diseases selected from the group consisting of allergic reaction, allergic rhinitis, anaphylactic and delayed hypersensitivity, psoriasis, atopic dermatitis, rheumatoid arthritis, Crohn´s disease, irritable bowel syndrome or male infertility may greatly benefit from the application of SGK1 modulators.

Prior to the application of SGK directed treatment it may be useful to examine the SGK1 status of a patient suffering from a mast cell activation dependent disorder and this is performed by measuring the up-regulated expression of SGK1, SGK2 or SGK3 in mast cells derived from tissue samples and specimens of the patient.

It is known in the art that the presence of a specific polymorph SGK1 protein variant predisposes for disease. Therefore a method for determining mast cell activation dependent disorders by measuring the up-regulated expression of a selected single nucleotide polymorph variant of SGK1 in mast cells derived from tissue samples and specimens of risk patients may be used.

SGK1 based diagnosis may be especially useful for evaluating human inflammatory disorder or human male infertility.

The present invention provides evidence that SGK is involved in the activation of mast cells. Among other aspects of this finding this allows the screening of an inhibitor of serum glucocorticoid inducible kinases (SGK) suitable for the inhibition of mast cell activation wherein the method comprises the following steps:

(i) providing a recombinant pre-activated phosphorylated SGK protein

(ii) providing an SGK substrate polypeptide together with ATP

(iii) providing an inhibitor of glucocorticoid inducible kinases, and

(iv) evaluating SGK activity by measuring phosphorylation of the substrate.

It is well understood that the SGK protein is selected from the group of SGK1, SGK2 or SGK3 or that alternatively or in addition the selected single nucleotide polymorph variant of SGK may used as well.

Furthermore the invention delivers a method for determining the progression, regression or onset of mast cell activation driven disorders by measuring the up-regulated expression and activation of SGK1, SGK2 or SGK3 and selected single nucleotide polymorph variants in isolated human tissue samples and specimens.

The expert understands that the claimed methodes are well suited for the diagnosis of disease, wherein the disease is selected from the group consisting of allergic reaction, allergic rhinitis, anaphylactic and delayed hypersensitivity, psoriasis, atopic dermatitis, rheumatoid arthritis, Crohn´s disease, irritable bowel syndrome or male infertility..

Furthermore the expert under stands that the compound selected with the claimed screening system or compounds selected form the lsted compound of Example 7 are well suited for the manufacture of a medicament for the inhibition of SGK1, SGK2 or SGK3 dependent mast cell activation and that such medicaments are useful for the treatment of disorders selected from the group consisting of allergic reaction, allergic rhinitis, anaphylactic and delayed hypersensitivity, psoriasis, atopic dermatitis, rheumatoid arthritis, Crohn´s disease, irritable bowel syndrome or male infertility.

Detailed description of the invention

The present study reveals a role of the serum and glucocorticoid inducible kinase SGK1 in the regulation of ion channel activity and function of mast cells. Distinct functional differences of mast cells are seen in gene targeted mice lacking SGK1 (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*+/+). Deficiency of SGK1 blunts the capacity of Ca^{2+} entry and reflects that I_{CRAC} is leading to subsequent impairment of Ca^{2+} dependent K^+ channel activation. Most

importantly, the *sgk1*$^{-/-}$ mice completely lack the early, mast cell dependent, response to TNCB, which induces delayed type hypersensitivity reactions (DTHRs).

The activation of Ca^{2+} channels is critically important for the regulation of mast cell function such as release of inflammatory mediators [Bradding et al., 2003; Buess et al., 1999; Dernick et al., 2003; Duffy et al., 2001a; Duffy et al., 2001b; Kahr et al., 2004; Mazurek et al., 1980; Stokes et al., 2004]. Activation of mast cells is further paralleled by activation of K^+ channels [Bradding et al., 2003; Bradding 2005; Duffy et al., 2005; Mark et al., 2004]. K^+ channels maintain the cell membrane potential, which is required for the function of I_{CRAC} [Parekh and Penner 1997].

With respect to the molecular identity of the channels that are involved in mast cell activation, PI3 kinase has been suggested to target the TRPV2 Ca^{2+} channel [Tseng et al., 2004]. SGK1 has previously been shown to increase the cell membrane abundance and activity of the Ca^{2+} channel TRPV5 [Embark et al., 2004; Palmada et al., 2005] and SGK1 may have a similar stimulating effect on TRPV2.

The molecular identity of the SGK1 regulated mast cell K^+ channels is similarly elusive. In other systems, SGK1 has been shown to activate several different K^+ channels [Baltaev et al., 2005; Embark et al., 2003; Embark et al., 2004; Gamper et al., 2002; Henke et al., 2004; Palmada et al., 2003; Ullrich et al., 2005; Wärntges et al., 2002; Yoo et al., 2003; Yun et al., 2002b].

SGK1 function is not limited to the regulation of ion channels but has a known influence on further transport systems, including the Na^+/H^+ exchanger NHE3 [Yun et al., 2002a; Yun 2003], several amino acid transporters [Boehmer et al., 2003b; Boehmer et al., 2003a], glucose transporters [Dieter et al., 2004] and the Na^+/K^+-ATPase [Henke et al., 2004; Setiawan et al., 2002; Verrey et al., 2003; Zecevic et al., 2004]. Deranged regulation of those transporters maybe the molecular basis for the functional defect of mast cells from SGK1 knockout animals.

SGK1 function is not limited to the basic functions of mast cells but participates in their regulation by hormones and mediators. SGK1 is genomically

unregulated by glucocorticoids [Firestone et al., 2003], mineralocorticoids [Chen et al., 1999; Naray-Fejes-Toth et al., 1999; Shigaev et al., 2000], cell shrinkage [Waldegger et al., 1997], gonadotropins [Alliston et al., 1997; Alliston et al., 2000; Gonzalez-Robayna et al., 2000; Richards et al., 1995], and TGFß [Lang et al., 2000; Waldegger et al., 1999]. The kinase is activated by IGF1 and insulin through the phosphatidyl-inositide 3 (PI3) kinase and phospho-inositide-dependent kinase PDK1 [Alessi et al., 1996; Alessi and Cohen 1998; Divecha et al., 1991; Gamper et al., 2002; Kobayashi and Cohen 1999; Kotani et al., 1994; Park et al., 1999].

In conclusion, SGK1 is critically important for the regulation of Ca^{2+} entry and K^+ channel activation of mast cells and the early phase of TNCB induced delayed type hypersensitivity reaction. The present observations thus disclose a completely novel regulator of mast cell function.

Detailed description of the results

As illustrated in Figure 1, mast cells cultured from bone marrow (BMCMC) express CD117, CD34 and FcεRI, i.e. the receptors typically expressed by mast cells. No significant difference in receptor abundance is observed between BMCMC from SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$).

The forward and side scatter in cultured mast cells from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice is shown in Figure 2. The forward scatter is slightly but significantly smaller in *sgk1*$^{-/-}$ BMCMC than in *sgk1*$^{+/+}$ BMCMC, pointing to a slightly smaller cell volume. Similarly, the side scatter is slightly but significantly smaller in *sgk1*$^{-/-}$ BMCMC than in *sgk1*$^{+/+}$ BMCMC, pointing to a slight decrease of granulation.

Furo-2 fluorescence reveals that Ca^{2+} entry following Ca^{2+} depletion is significantly smaller in *sgk1*$^{-/-}$ BMCMC than in *sgk1*$^{+/+}$ BMCMC, pointing to defective activation of the Ca^{2+} release activated Ca^{2+} channel I_{CRAC} in *sgk1*$^{-/-}$ mice.

Patch clamp reveals the activation of Ca^{2+} sensitive K^+ channels by IgE-DNP in *sgk1*$^{+/+}$ BMCMC, an effect lacking completely in *sgk1*$^{-/-}$ BMCMC. The channels are inhibited by clotrimazole, a known blocker of Ca^{2+} sensitive K^+ channels. Treatment of the cells with the Ca^{2+} ionophore ionomycin (1 µM) leads to activation of the K^+ channels to a similar extent in *sgk1*$^{+/+}$ BMCMC and *sgk1*$^{-/-}$ BMCMC, indicating that the Ca^{2+} sensitive K^+ channels are similarly expressed and similarly sensitive to activation by Ca^{2+} in *sgk1*$^{+/+}$ BMCMC and *sgk1*$^{-/-}$ BMCMC. Thus, the defective regulation of K^+ channels presumably results from blunted increase of cytosolic Ca^{2+} activity.

Further studies have been performed to explore whether the impaired stimulation of Ca^{2+} in *sgk1*$^{-/-}$ BMCMC affects the *in vivo* function of mast cells. To this end, Trinitrochlorobenzene -induced contact hypersensitivity reaction has been tested in both, *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice. As illustrated in Figs. 5 and 6, the early ear swelling following stimulation with Trinitrochlorobenzene (TNCB) was completely lacking in *sgk1*$^{-/-}$ mice, an observation indeed pointing to severe impairment of mast cell function *in vivo*.

Further examples

Examples 1: Dexamethasone treatment of mice

Mice lacking SGK1 (*sgk1*$^{-/-}$) have been generated as described previously [Wulff et al., 2002]. A conditional targeting vector was generated from a 7-kb fragment encompassing the entire transcribed region on 12 exons. The neomycin resistance cassette was flanked by two loxP sites and inserted into intron 11. Exons 4–11, which code for the Sgk1 kinase domain, were "floxed" by inserting a third loxP site into intron 3. Targeted R1 ES cells were transiently transfected with Cre recombinase. A clone with a recombination between the first and third loxP site (type I recombination) was injected into C57BL/6 blastocytes. Male chimeras were bred to 129/SvJ females. Heterozygous *sgk1*-deficient mice were backcrossed to 129/SvJ wild-type

mice for two generations and then intercrossed to generate homozygous *sgk1*$^{-/-}$ and *Sgk1*$^{+/+}$ littermates. The animals were genotyped by PCR using standard methods. The study has been performed in female (n=15) and male (n=5) sgk1$^{+/+}$ and sgk1$^{-/-}$ (6-7 weeks old) mice.

For analysis of dexamethasone effects, *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice were injected with dexamethasonephosphate disodiumsalt (Sigma, Taufkirchen, Germany; dissolved in 0.9% saline) at a dose of 10µg/g BW for four consecutive days at 8 pm. *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice injected with 0.9% saline alone served as controls. Mice had free access to a standard mouse diet (Altromin diet 1310, Heidenau, Germany) and tap water. On the 4th day of dexamethasone treatment, the animals were fasted 16 hours on wire grids prior to the experiments with free access to tap water. Where specified the KCNQ1 K^+ channel inhibitor 293B was applied as a single bolus intravenous injection at a dose of 5µg/g BW 30 minutes before the experiment.

Example 2: Quantitative real-time PCR was used to determine the effect of glucocorticoids on SGK1 transcript levels, gastric tissue was quickly removed and frozen in liquid nitrogen. Automated disruption and homogenization of frozen tissue was performed using the MagNa Lyser Instrument™ (Roche Diagnostics, Mannheim, Germany). For each sample one-way special tubes were filled with ceramic beads, 20-30 mg of frozen tissue and 600 µl of RLT-buffer (Qiagen, Hilden, Germany). Cleared cell lysate was transferred for further RNA purification process (RNAeasy Mini Kit, Qiagen, Hilden, Germany). Subsequently 1 µg of total RNA was reverse transcribed to cDNA utilizing the reverse transcription system (Bioscience, USA) with oligo(dT) primers according to the manufacturer's protocol. To determine mSGK1 mRNA levels, quantitative real-time PCR with the LightCycler System™ (Roche Diagnostics, Mannheim, Germany) was established. PCR reactions for mSGK1 were performed in a final volume of 20 µl containing 2 µl cDNA, 2.4 µl $MgCl_2$ (3 µM), 1 µl primermix (0.5 µM of both primers), 2 µl cDNA Master SybrGreen I mix (Roche Molecular Biochemicals, Mannheim, Germany) and 12.6 µl DEPC treated water. The transcript levels of the

housekeeping gene mGAPDH were determined in each sample using a commercial primer kit (Search LC, Heidelberg, Germany). PCR reactions for GAPDH were performed in a final volume of 20 µl containing 2 µl cDNA, 2 µl primer mix (Search LC, Heidelberg, Germany), 2 µl cDNA Master Sybr Green I mix (Roche Molecular Biochemicals, Mannheim, Germany) and 14 µl DEPC treated water.

Amplification of the target DNA was performed during 35 cycles of 95°C for 10s, 68°C for 10s and 72°C for 16s, each with a temperature transition rate of 20°C/s and a secondary target temperature of 58°C with a step size of 0.5°C. Melting curve analysis was performed at 95°C 0s, 58°C 10s, 95°C 0s to determine melting temperatures of primer dimers and the specific PCR products. Melting curve analysis confirmed the amplified products, which were then separated on 1.5% agarose gels to confirm the expected size (406 bp). Finally, results were calculated as a ratio of the target vs. house keeping gene transcripts.

The following primers for mSGK1 (Gene bank No.: NM_011361) were used:

mSGK1 sense: 5' TGT CTT GGG GCT GTC CTG TAT G 3'

mSGK1 antisense: 5' GCT TCT GCT GCT TCC TTC ACA C 3'

Example 3: Culture and analysis of bone marrow mast cells

Bone marrow derived cultured mast cells (BMMCs) were isolated from bone marrow of 12 weeks old male *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ naive mice. The cells were cultured for 4 weeks in RPMI 1640 (GIBCO, Carlsbad) containing 10% FCS, 1% penicillin/streptomicin, 0.5 µg IL-3 (RD systems, Wiesbaden-Nordenstadt) and 1µg c-kit ligand (SCF, BIOZOL, Eching, Germany). The maturation of the cells was confirmed by staining the cells against: FceRI (e Bioscience/NatuTec Gmbh, Frankfurt), CD117 kit (BD Pharmingen, Heidelberg), and CD34 (BD Pharmingen, Heidelberg) molecules and analysis by Flow Cytometry (FACS Calibur, Becton Dickinson).BMMCs were activated by sensitization with monoclonal mouse IgE Ab (1:100, 12µg/ml/ $10x^6$ cells, clone SPE-7, Sigma Aldrich, Munich) overnight in culture medium and

challenged accutely with (100ng/ml) DNP-HSA (dinitrophenyl –human serum albumin, Sigma Aldrich, Munich) [Bradding et al., 2003].

Example 4: Trinitrochlorobenzen (TNCB)-induced contact hypersensitivity reaction (CHSRs)

TNCB (Sigma Aldrich, Munich) was used to induce delayed type hypersensitivity reactions (DTHRs), which are strictly dependent on hapten-specific, type 1 memory T cells and are associated with a strong infiltrate of polymorphic neutrophils PMNs [Biedermann et al., 2000; Pichler et al., 2005]. These memory T cells lead to CHSRs when the hapten is applied to the skin of sensitized mice [Asada et al., 1997]. BALB/c mice were sensitized with 5% TNCB (n=6) (80µl of a 4:1 mixture of acetone/olive oil) at the shaved abdomen, size: 2 per 2 cm. 1 week later, mice were challenged with 1% TNCB (20 µl of a 1:9 mixture of acetone/olive oil) on both sides of the ear. In the 5% TNCB solution acetone is an irritant, whereas it is used solely as a solvent in the 1% TNCB solution. Specific ear swelling was determined by measuring ear thickness with a micrometer (Oditest ®;Kroeplin) before and 4, 8, 12 and 48 h after TNCB challenge. Data are expressed as a change in ear swelling comparing to thickness before treatment, (delta µm). In addition, ear tissue was collected 48 h after TNCB challenge and sections were stained with hematoxylin and eosin.

Example 5: Patch clamp experiments

Patch clamp experiments have been performed at room temperature in voltage-clamp, fast-whole-cell mode according to Hamill et al. [Hamill et al., 1981]. The cells were continuously superfused through a flow system inserted into the dish. The bath was grounded via a bridge filled with NaCl Ringer solution. Borosilicate glass pipettes (8-12 MOhm tip resistance; GC 150 TF-10, Clark Medical Instruments, Pangbourne, UK) manufactured by a microprocessor-driven DMZ puller (Zeitz, Augsburg, Germany) were used in

combination with a MS314 electrical micromanipulator (MW, Märzhäuser, Wetzlar, Germany). The currents were recorded by an EPC-9 amplifier (Heka, Lambrecht, Germany) using Pulse software (Heka) and an ITC-16 Interface (Instrutech, Port Washington, N.Y., USA). Whole-cell currents were determined at eleven successive 700-ms square pulses from the -20 mV holding potential to potentials between -100 mV and +80 mV. The current values were 3 kHz low-pass filtered.

The pipette solution contained (in mM): 115 Na-gluconate, 10 NaCl, 1 MgATP, 1 EGTA, and 5 HEPES/NaOH (pH 7.4) and was used in combination with NaCl and Cl^--free Ringer solutions (see above).

The offset potentials between both electrodes were zeroed before sealing. The potentials were corrected for liquid junction potentials as estimated according to Barry & Lynch [Barry and Lynch 1991]. The original whole-cell current traces are depicted without filtering (acquisition frequency of 5 kHz) and currents of the individual voltage square pulses are superimposed. The applied voltages refer to the cytoplasmic face of the membrane with respect to the extracellular space. The inward currents, defined as flow of positive charge from the extracellular to the cytoplasmic membrane face, are negative currents and depicted as downward deflections of the original current traces.

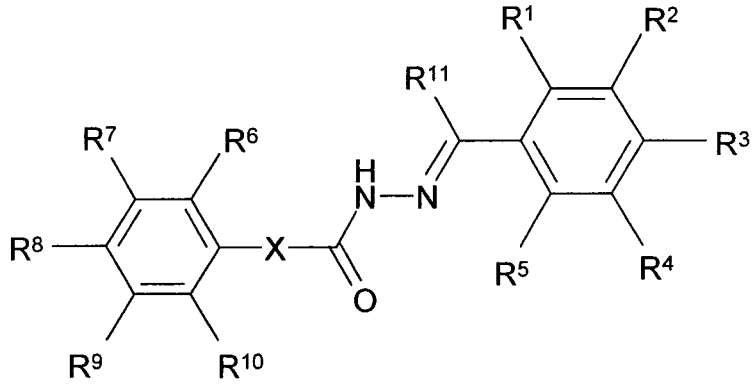
Example 6: Measurement of intracellular Ca^{2+}

Fura-2 fluorescence was utilized for cytosolic Ca^{2+} determinations. Intracellular Ca^{2+} measurements were performed as described [Tanneur et al., 2002]. Retinoblastoma cells were loaded with Fura-2 (2.5µM- Molecular Probes, Goettingen, Germany) for 30 minutes at 37°C. Fluorescence measurements were carried out with an inverted phase-contrast microscope (Axiovert 100, Zeiss, Oberkochen, Germany). Cells were excited alternatively at 340 and 380 nm and the light was deflected by a dichroic mirror into the objective (Fluar 40×/1.30 oil, Zeiss, Oberkochen, Germany). Emitted fluorescence intensity was recorded at 505 nm and data acquisition was performed by Axon Imaging Workbench (Axon Instruments, Foster City,

USA). Experiments were made prior to, during and following exposure to nominally Ca^{2+} free solution (5 mM EGTA added). In the absence of Ca^{2+} the intracellular Ca^{2+} stores were depleted by inhibition of the vesicular Ca^{2+} pump by thapsigargin (1 µM, Molecular Probes).

Example 7: SGK1 inhibiting and modulating compounds

7.1. Compounds of the general formula I and pharmaceutical useful derivates, salts, solutions and stereoisomeres thereof including mixtures.



I

wherein

R^1, R^5 is either H, OH, OA, OAc or Methyl,

R^2, R^3, R^4, R^6, R^7, R^8, R^9, R^{10} is either H, OH, OA, OAc, OCF_3, Hal, NO_2, CF_3, A, CN, OSO_2CH_3, SO_2CH_3, NH_2 or COOH,

R^{11} H or CH_3, A Alkyl with 1, 2, 3 or 4 C-atoms,

X CH_2, CH_2CH_2, OCH_2 or -CH(OH)-, Hal, F, Cl, Br or I

Compound according to formula I selected from the following group of compounds:

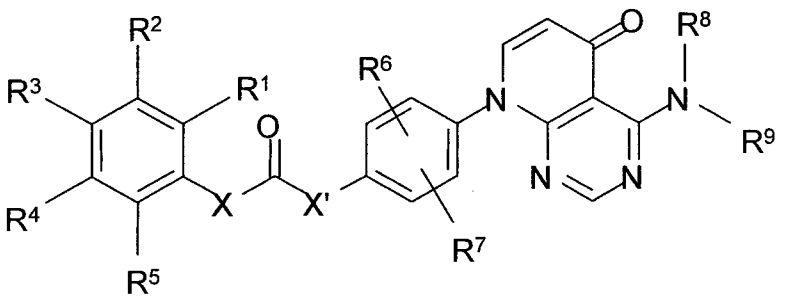
(3-Hydroxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Hydroxy-phenyl)-acidic acid-[1-(4-hydroxy-2-methoxy-phenyl)-ethyliden]-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid.

Phenylacidic acid-(3-fluor-4-hydroxy-benzyliden)-hydrazid,
(4-Hydroxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,

(3,4-Dichlor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
m-Tolyl-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
o-Tolyl-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(2-Chlor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Chlor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(4-Fluor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(2-Chlor-4-fluor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Fluor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(4-hydroxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(4-hydroxy-2,6-dimethyl-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(3-fluor-4-hydroxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-[1-(4-hydroxy-2-methoxy-phenyl)-ethyliden]-hydrazid,
(3-Methylsulfonyloxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3,5-Dihydroxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Fluor-phenyl)-acidic acid-(3-fluor-4-hydroxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(4-acetoxy-2-methoxy-benzyliden)-hydrazid,
(3-Trifluormethyl-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
3-(3-Methoxy-phenyl)-propionsäure-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(2,4-dihydroxy-benzyliden)-hydrazid,
(3-Methoxy-phenoxy)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Nitro-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(5-chlor-2-hydroxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(2-hydroxy-5-nitro-benzyliden)-hydrazid,
2-Hydroxy-2-phenyl-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(2-ethoxy-4-hydroxy-benzyliden)-hydrazid,
(3-Brom-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,

(3-Methoxy-phenyl)-acidic acid-[1-(4-hydroxy-phenyl)-ethyliden]-hydrazid,
(3,5-Difluor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Hydroxy-phenyl)-acidic acid-(4-hydroxy-2-methyl-benzyliden)-hydrazid,
(3-Hydroxy-phenyl)-acidic acid-(2-ethoxy-4-hydroxy-benzyliden)-hydrazid,
(3-Hydroxy-phenyl)-acidic acid-(2-methoxy-4-hydroxy-6-methyl-benzyliden)-hydrazid,
(2-Fluor-phenyl)-acidic acid-(2-methoxy-4-hydroxy-benzyliden)-hydrazid

7.2. Compounds of the general formula II and pharmaceutical useful derivates, salts, solutions and stereoisomeres thereof including mixtures.



II

wherein

R^1, R^2, R^3, R^4, R^5	is either H, A, OH, OA, Alkenyl, Alkinyl, NO_2, NH_2, NHA, NA_2, Hal, CN, COOH, COOA, -OHet, -O-Alkylen-Het, -O-Alkylen-NR^8R^9 or $CONR^8R^9$, two groups selected from R^1, R^2, R^3, R^4, R^5 or as well -O-CH_2-CH_2-, -O-CH_2-O- or -O-CH_2-CH_2-O-,
R^6, R^7	is either H, A, Hal, OH, OA or CN,
R^8, R^9	is either H or A,
Het	Is a saturated or unsaturated heterocycle with 1 to 4 N-, O- and/or S-atoms, substituted by one or several Hal, A, OA, COOA, CN or Carbonyloxigen (=O)
A	Alkyl with 1 to 10 C-atoms, wherein 1-7 H-atoms may be replaced by F and/or Chlorine,
X, X'	is either NH or is missing
Hal	F, Cl, Br or I

Compound according to formula II selected from the following group of compounds:

1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2-fluor-5-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(4-chlor-5-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,4-difluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,6-difluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(3-fluor-5-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(4-fluor-5-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(4-methyl-5-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,3,4,5,6-pentafluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,4-dibrom-6-fluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2-fluor-6-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2-fluor-5-methyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,3,4-trifluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(4-brom-2,6-difluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2-fluor-3-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-(1-tert.-butyloxycarbonyl-piperidin-4-yl)-phenyl]-urea,
N-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-2,4-dichlor-benzamid,

N-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-4-chlor-5-trifluormethyl-benzamid,
N-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-2-fluor-5-trifluormethyl-benzamid,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[3-chlor-5-trifluormethyl-2-(piperidin-4-yloxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[(2-fluor-5-(2-dimethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[5-fluor-2-(piperidin-4-yloxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-chlor-5-trifluormethyl-2-(piperidin-4-yloxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-(piperidin-4-yloxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-fluor-5-(2-diethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-fluor-5-[2-(piperidin-1-yl)-ethoxy]-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-fluor-2-(2-dimethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-fluor-2-(2-diethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[3-chlor-4-[2-(morpholin-4-yl)-ethoxy]-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-fluor-2-[2-(morpholin-4-yl)-ethoxy]-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[3-chlor-4-(2-dimethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[3-chlor-4-(2-diethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-chlor-2-(2-dimethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-chlor-5-(2-diethylamino-ethoxy)-phenyl]-urea,

Example 8: SGK1 nucleotide polymorphism

SGK1 nucleotide polymorphism is demonstrated by the sequences ...aattacattgCgcaacccag.., whereas the nucleotide sequence representing a another population is....aattacattgTgcaacccag....Both sequences are available through accession number GI 2463200 Position 2071.

The exon 8 sequences of facultative patients with mast cell overactivity are either homozygot ..tactgaCttcggact..or....tactgaTttcggact....or heterozygot .tactgaCttcggact...and...tactgaTttcggact .. The sequences are available through accession number NM_005627.2, Position 777.

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Figure legends

Figure 1: Cell surface receptor expression in cultured mast cells from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice

FACS analysis illustrating CD117, CD34 and FcεRI expression in cultured bone marrow mast cells (BMCMC) from SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$).

Figure 2: Forward and side scatter in cultured mast cells from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice.

Arithmetic means (± SEM; n = 7) of forward scatter (FSC, left panel) and side scatter (SSC, right panel) of cultured bone marrow mast cells (BMCMC) in arbitrary units (a.u.) from SGK1 knockout mice (*sgk1*$^{-/-}$, black bars) and their wild type littermates (*sgk1*$^{+/+}$, white bars).

Figure 3: Ca^{2+} entry into cultured mast cells from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice.

Arithmetic means (± SEM; n = 21) of increase of Fura-2 fluorescence in arbitrary units (a.u.) reflecting cytosolic Ca^{2+} into cultured bone marrow mast cells (BMCMC) from SGK1 knockout mice (*sgk1*$^{-/-}$, black bars) and their wild type littermates (*sgk1*$^{+/+}$, white bars).

Figure 4: Activation of Ca^{2+} sensitive K^+ channels in cultured mast cells from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice.

A. Mean I-V relationships (± SEM, n = 4) of currents in cultured bone marrow mast cells (BMCMC) from SGK1 knockout mice (*sgk1*$^{-/-}$, right panel) and their wild type littermates (*sgk1*$^{+/+}$, left panel) prior to (open circles) and following (closed triangles) activation with IgE-DNP in the absence (closed triangles) and presence (closed squares) of clotrimazole.

B. Mean whole-cell conductance (± SEM, n = 4-6) obtained from cultured bone marrow mast cells (BMCMC) from SGK1 knockout mice (*sgk1*$^{-/-}$, black bars) and their wild type littermates (*sgk1*$^{+/+}$, white bars) prior to (control) and following exposure to IgE-DNP (IgE) and/or clotrimazole (CTZ) * indicates significant difference ($p<0.05$; ANOVA).

Figure 5: Ear tissue from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice prior to and following Trinitrochlorobenzene -induced contact hypersensitivity reaction.

Sections of ear tissue from SGK1 knockout mice (*sgk1*$^{-/-}$, left panel) and their wild type littermates (*sgk1*$^{+/+}$, right panel) 8 hours following (lower panels) stimulation with Trinitrochlorobenzene (TNCB).

Figure 6: Ear swelling of *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice prior to and following Trinitrochlorobenzene -induced contact hypersensitivity reaction.

Time course (arithmetic means ± SEM, n = 6) of the swelling (in µm) of right (left panel) and left (right panel) ears from SGK1 knockout mice (*sgk1*$^{-/-}$, closed circles) and their wild type littermates (*sgk1*$^{+/+}$, open circles) following stimulation with Trinitrochlorobenzene (TNCB). * indicates significant difference ($p<0.05$; ANOVA).

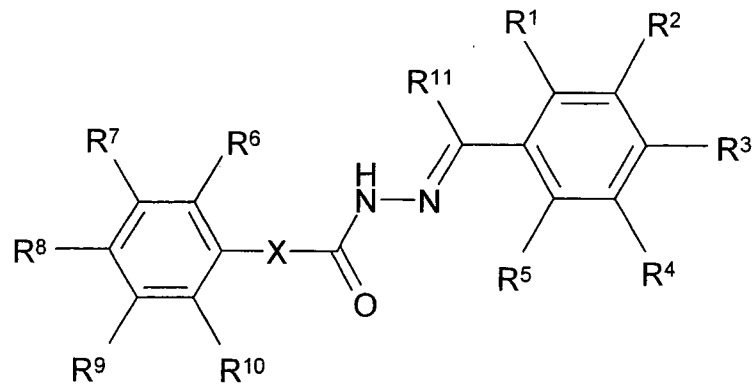
Claims

1. A method for the screening of an inhibitor of serum glucocorticoid inducible kinases (SGK) suitable for the inhibition of mast cell activation wherein the method comprises the following steps:
(i) providing a recombinant pre-activated phosphorylated SGK protein
(ii) providing an SGK substrate polypeptide together with ATP
(iii) providing an inhibitor of glucocorticoid inducible kinases, and
(iv) evaluating SGK activity by measuring phosphorylation of the substrate.

2. A method according to claim 1, wherein the SGK protein is selected from the group of SGK1, SGK2 or SGK3.

3. A method according to claim 2, wherein SGK1, SGK2 or SGK3 represents a selected single nucleotide polymorph variant.

4. A method according to claims 1-3, wherein the SGK inhibitors have the general formula:



I

wherein

R^1, R^5 is either H, OH, OA, OAc or Methyl,

R^2, R^3, R^4, R^6, R^7, R^8, R^9, R^{10} is either
H, OH, OA, OAc, OCF_3, Hal, NO_2, CF_3, A, CN, OSO_2CH_3, SO_2CH_3, NH_2 or COOH,

R^{11} H or CH_3,

A Alkyl with 1, 2, 3 or 4 C-atoms,

X CH_2, CH_2CH_2, OCH_2 or -CH(OH)-,

Hal F, Cl, Br or I

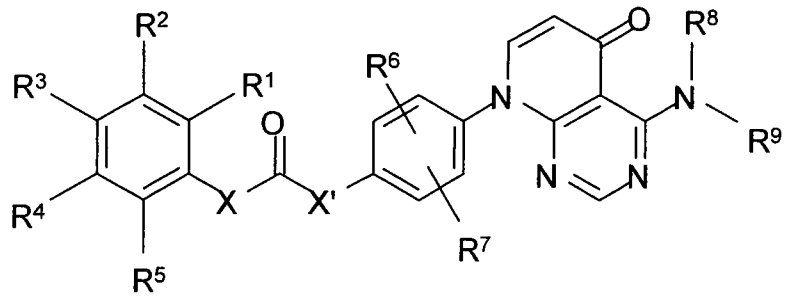
and pharmaceutical useful derivates, salts, solutions and stereoisomeres thereof including mixtures.

5. A method according to claim 5 wherein the the SGK inhibitor is selected from the following group of compounds:

(3-Hydroxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Hydroxy-phenyl)-acidic acid-[1-(4-hydroxy-2-methoxy-phenyl)-ethyliden]-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
Phenylacidic acid-(3-fluor-4-hydroxy-benzyliden)-hydrazid,
(4-Hydroxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3,4-Dichlor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
m-Tolyl-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
o-Tolyl-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(2-Chlor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Chlor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(4-Fluor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(2-Chlor-4-fluor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Fluor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(4-hydroxy-benzyliden)-hydrazid, (3-Methoxy-phenyl)-acidic acid-(4-hydroxy-2,6-dimethyl-benzyliden)-hydrazid, (3-Methoxy-phenyl)-acidic acid-(3-fluor-4-hydroxy-benzyliden)-

hydrazid, (3-Methoxy-phenyl)-acidic acid-[1-(4-hydroxy-2-methoxy-phenyl)-ethyliden]-hydrazid,
(3-Methylsulfonyloxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3,5-Dihydroxy-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Fluor-phenyl)-acidic acid-(3-fluor-4-hydroxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(4-acetoxy-2-methoxy-benzyliden)-hydrazid,
(3-Trifluormethyl-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
3-(3-Methoxy-phenyl)-propionsäure-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(2,4-dihydroxy-benzyliden)-hydrazid,
(3-Methoxy-phenoxy)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Nitro-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(5-chlor-2-hydroxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(2-hydroxy-5-nitro-benzyliden)-hydrazid,
2-Hydroxy-2-phenyl-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-(2-ethoxy-4-hydroxy-benzyliden)-hydrazid,
(3-Brom-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Methoxy-phenyl)-acidic acid-[1-(4-hydroxy-phenyl)-ethyliden]-hydrazid,
(3,5-Difluor-phenyl)-acidic acid-(4-hydroxy-2-methoxy-benzyliden)-hydrazid,
(3-Hydroxy-phenyl)-acidic acid-(4-hydroxy-2-methyl-benzyliden)-hydrazid,
(3-Hydroxy-phenyl)-acidic acid-(2-ethoxy-4-hydroxy-benzyliden)-hydrazid,
(3-Hydroxy-phenyl)-acidic acid-(2-methoxy-4-hydroxy-6-methyl-benzyliden)-hydrazid,
(2-Fluor-phenyl)-acidic acid-(2-methoxy-4-hydroxy-benzyliden)-hydrazid

6. A method according to claim 1-3, wherein the SGK inhibitors have the general formula:



II

wherein

R^1, R^2, R^3, R^4, R^5 is either H, A, OH, OA, Alkenyl, Alkinyl, NO_2, NH_2, NHA, NA_2, Hal, CN, COOH, COOA, -OHet, -O-Alkylen-Het, -O-Alkylen-NR^8R^9 or $CONR^8R^9$, two groups selected from R^1, R^2, R^3, R^4, R^5 or as well -O-CH_2-CH_2-, -O-CH_2-O- or -O-CH_2-CH_2-O-,

R^6, R^7 is either H, A, Hal, OH, OA or CN,

R^8, R^9 is either H or A,

Het is a saturated or unsaturated heterocycle with 1 to 4 N-, O- and/or S-atoms, substituted by one or several Hal, A, OA, COOA, CN or Carbonyloxigen (=O)

A Alkyl with 1 to 10 C-atoms, wherein 1-7 H-atoms may be replaced by F and/or Chlorine,

X, X' is either NH or is missing Hal, F, Cl, Br or I

and pharmaceutical useful derivates, salts, solutions and stereoisomeres thereof including mixtures.

7. A method according to claim 6 wherein the SGK inhibitor is selected from the following group of compounds:

1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2-fluor-5-trifluormethyl-phenyl)-urea,

1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(4-chlor-5-trifluormethyl-phenyl)-urea,

1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,4-difluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,6-difluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(3-fluor-5-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(4-fluor-5-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(4-methyl-5-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,3,4,5,6-pentafluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,4-dibrom-6-fluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2-fluor-6-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2-fluor-5-methyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2,3,4-trifluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(4-brom-2,6-difluor-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-(2-fluor-3-trifluormethyl-phenyl)-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-(1-tert.-butyloxycarbonyl-piperidin-4-yl)-phenyl]-urea,
N-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-2,4-dichlor-benzamid,
N-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-4-chlor-5-trifluormethyl-benzamid,
N-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-2-fluor-5-trifluormethyl-benzamid,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[3-chlor-5-trifluormethyl-2-(piperidin-4-yloxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[(2-fluor-5-(2-dimethylamino-ethoxy)-phenyl]-urea,

1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[5-fluor-2-(piperidin-4-yloxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-chlor-5-trifluormethyl-2-(piperidin-4-yloxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-(piperidin-4-yloxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-fluor-5-(2-diethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-fluor-5-[2-(piperidin-1-yl)-ethoxy]-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-fluor-2-(2-dimethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-fluor-2-(2-diethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[3-chlor-4-[2-(morpholin-4-yl)-ethoxy]-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-fluor-2-[2-(morpholin-4-yl)-ethoxy]-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[3-chlor-4-(2-dimethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[3-chlor-4-(2-diethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[4-chlor-2-(2-dimethylamino-ethoxy)-phenyl]-urea,
1-[4-(4-Amino-5-oxo-5*H*-pyrido[2,3-*d*]pyrimidin-8-yl)-phenyl]-3-[2-chlor-5-(2-diethylamino-ethoxy)-phenyl]-urea,

8. A method for determining the progression, regression or onset of mast cell activation driven disorders by measuring the up-regulated expression and activation of SGK1, SGK2 or SGK3 in isolated human tissue samples and specimens.

9. A method according to claim 8, wherein SGK1, SGK2 or SGK3 represents a selected single nucleotide polymorph variant.

10. A method according to claim 9 for the diagnosis of disease, wherein the disease is selected from the group consisting of allergic reaction, allergic rhinitis, anaphylactic and delayed hypersensitivity, psoriasis, atopic dermatitis, rheumatoid arthritis, Crohn´s disease, irritable bowel syndrome or male infertility..

11. A method according to claim 10, wherein the SGK1, SGK2 or SGK3 represents a selected single nucleotide polymorph variant.

12. Use of a compound as specified in any one of the claims 4-7 for the manufacture of a medicament for the inhibition of SGK1, SGK2 or SGK3 dependent mast cell activation.

13. Use of a compound according to claim 12 for the manufacture of a medicament for the treatment of disorders selected from the group consisting of allergic reaction, allergic rhinitis, anaphylactic and delayed hypersensitivity, psoriasis, atopic dermatitis, rheumatoid arthritis, Crohn´s disease, irritable bowel syndrome or male infertility.

Figure 1

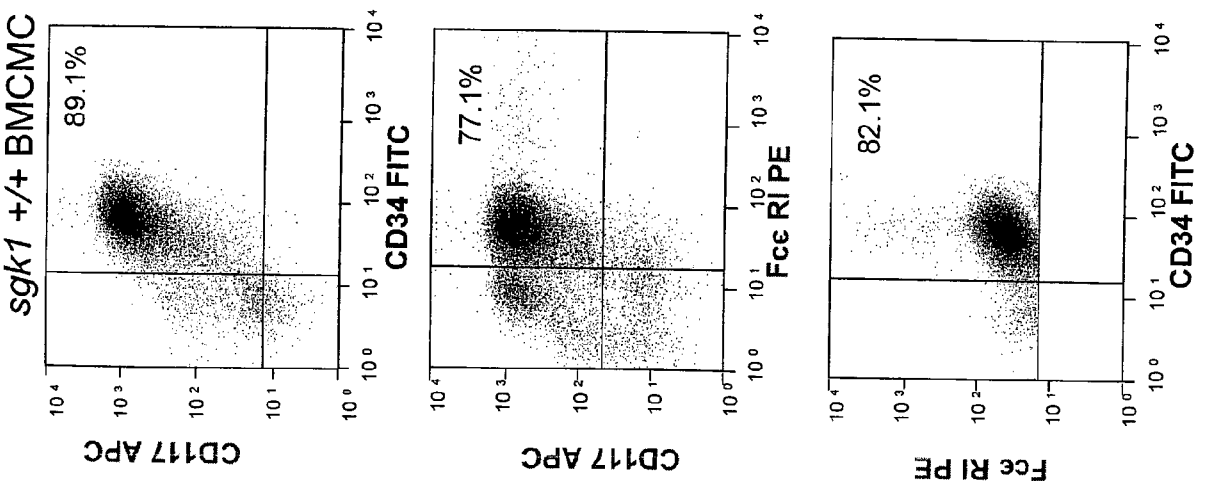

sgk1 +/+ BMCMC
89.1%
CD117 APC
CD34 FITC
77.1%
CD117 APC
Fcε RI PE
82.1%
Fcε RI PE
CD34 FITC
sgk1 -/- BMCMC
87.5%
CD117 APC
CD34 FITC
67%
CD117 APC
Fcε RI PE
70.5%
Fcε RI PE
CD34 FITC

Figure 2


SSC (a.u.)
250
200
150
100
50
*
FSC (a.u.)
450
400
350
300
250
200
150
100
50
0
*
□ sgk1+/+
■ sgk1-/-

Figure 3

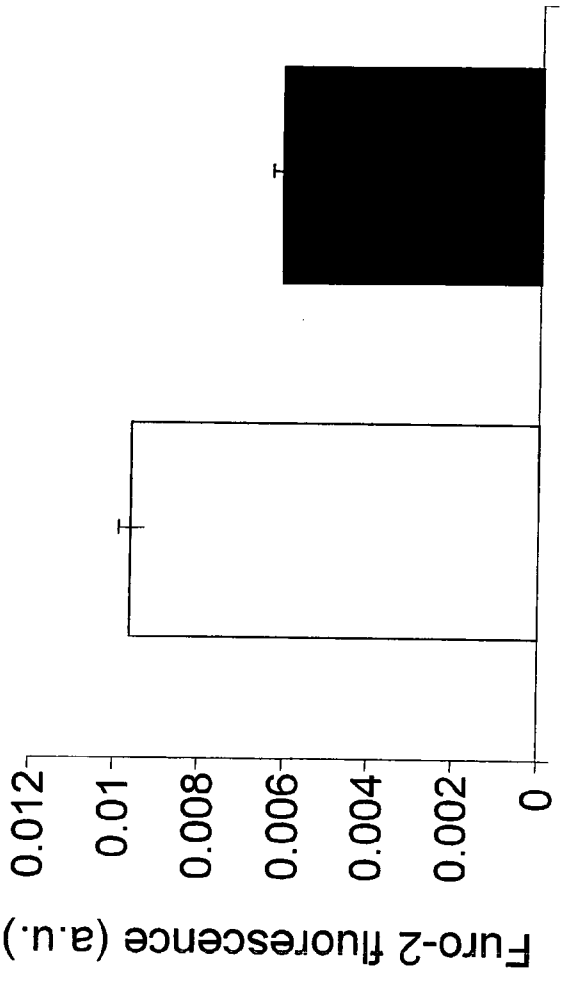

□ sgk1+/+
■ sgk1 -/-
Fura-2 fluorescence (a.u.)
0.012
0.01
0.008
0.006
0.004
0.002
0

Figure 4

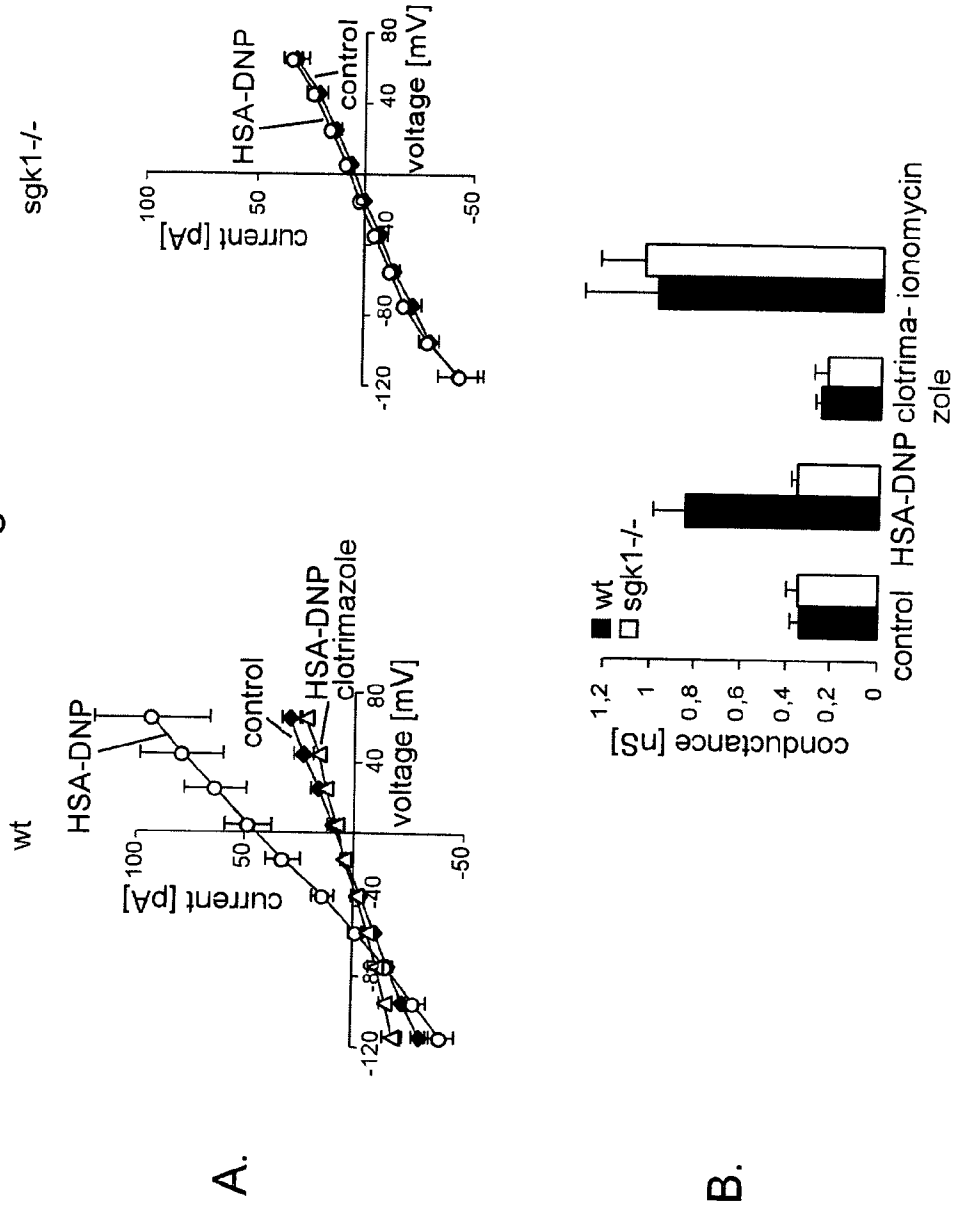
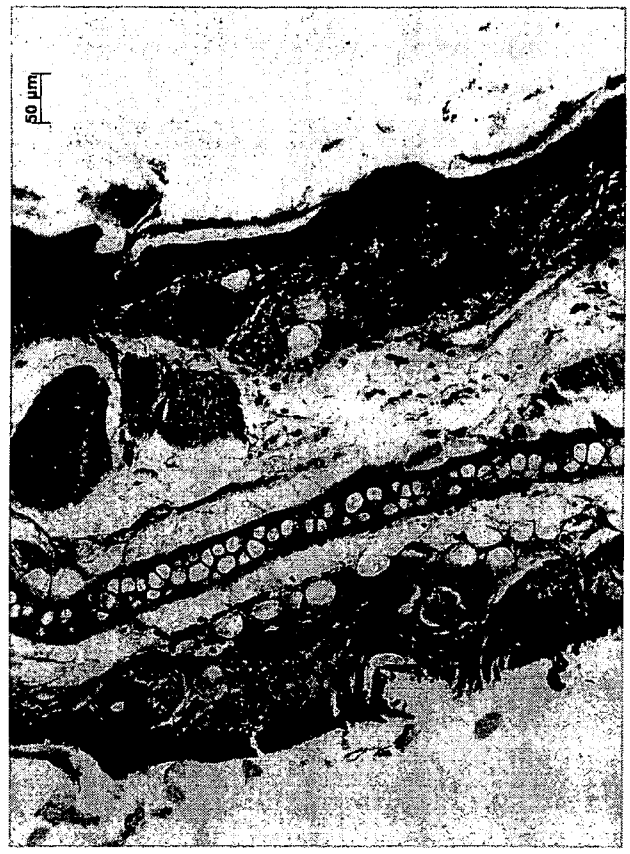
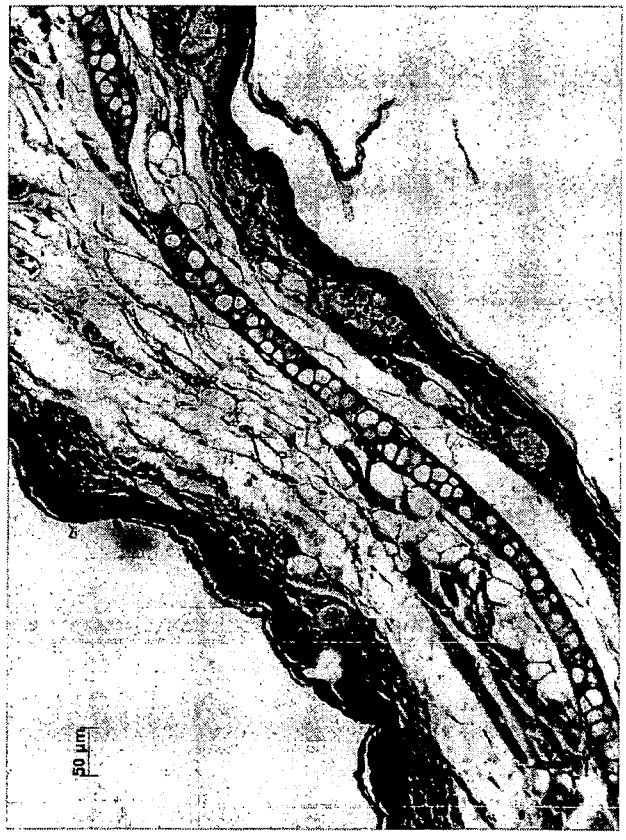

A.
wt
HSA-DNP
control
HSA-DNP
clotrimazole
current [pA]
voltage [mV]
sgk1-/-
HSA-DNP
control
current [pA]
voltage [mV]
B.
wt
sgk1-/-
conductance [nS]
control
HSA-DNP
clotrima-zole
ionomycin

Figure 5

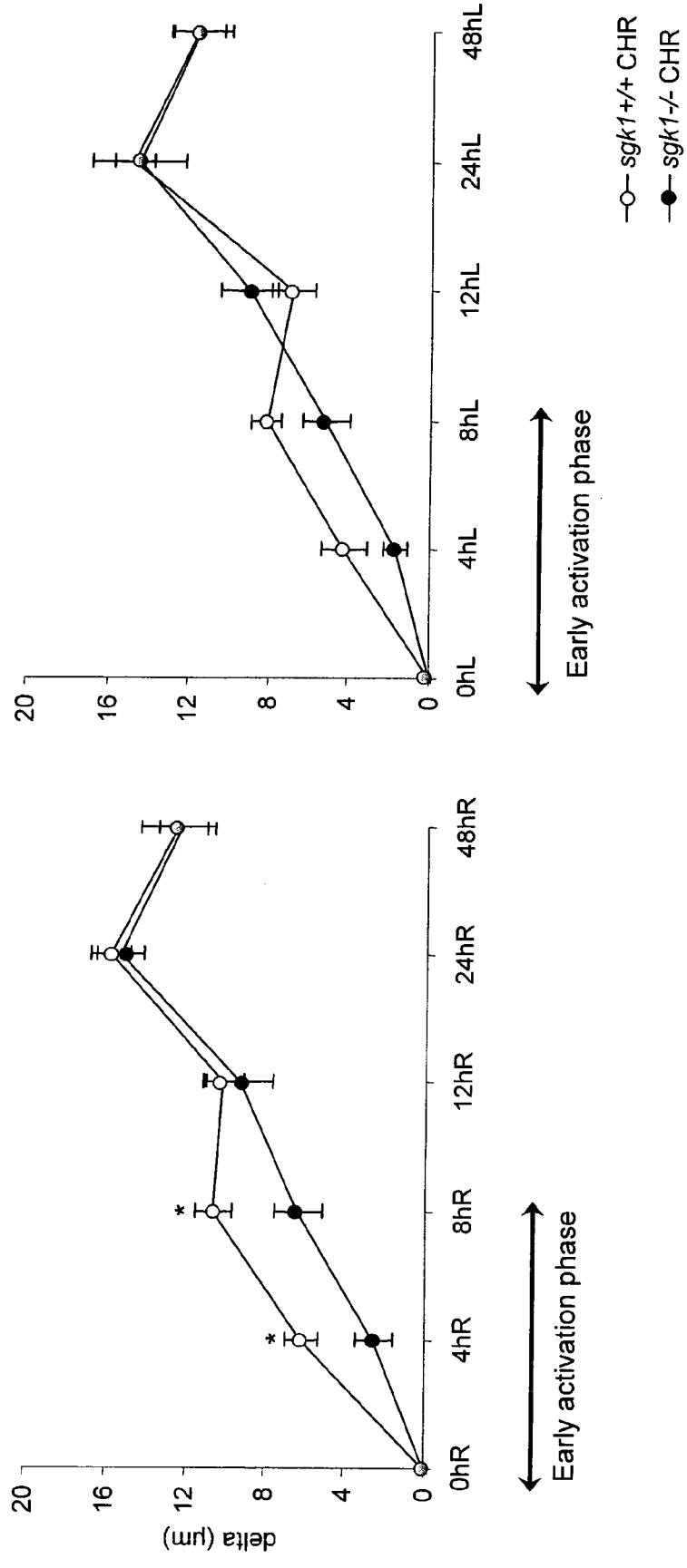


sgk1 +/+ CHR



sgk1 -/- CHR

Figure 6


delta (µm)
20
16
12
8
4
0
0hR
4hR
8hR
12hR
24hR
48hR
*
*
Early activation phase
0hL
4hL
8hL
12hL
24hL
48hL
Early activation phase
sgk1+/+ CHR
sgk1-/- CHR

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/003535

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 A61K31/165

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 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, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/011681 A (SMITHKLINE BEECHAM CORP [US]; DREWRY DAVID HAROLD [US]; LINN JAMES AND) 10 February 2005 (2005-02-10) page 66 - page 69	1-3,8-13
X	DE 103 46 913 A1 (MERCK PATENT GMBH [DE]) 4 May 2005 (2005-05-04) pages 2-6 - pages 11-20	4,5,8-13
X	WO 2005/094796 A (MERCK PATENT GMBH [DE]; LANG FLORIAN [DE]) 13 October 2005 (2005-10-13) page 5; claims 1-7; example 4	1-13

-/--

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

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2007	09/08/2007

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

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/003535

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WYMANN M P ET AL: "Phosphoinositide 3-kinase in disease: timing, location, and scaffolding" CURRENT OPINION IN CELL BIOLOGY, CURRENT SCIENCE, LONDON, GB, vol. 17, no. 2, April 2005 (2005-04), pages 141-149, XP004869317 ISSN: 0955-0674 figure 1	1-13

INTERNATIONAL SEARCH REPORT

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International application No
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