(54) Title: TRIAZOLE DERIVATIVES AND THEIR USE AS PDE4 ACTIVATORS
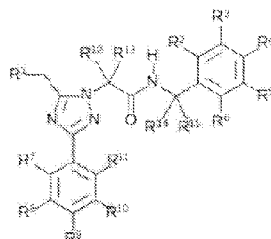
(I)

(57) **Abstract**: Compounds of Formula (I), which are activators of long form cyclic nucleotide phosphodiesterase-4 (PDE4) enzymes, are provided. Methods and uses of these compounds for the treatment or prevention of disorders requiring a reduction of second messenger responses mediated by cyclic 3',5'-adenosine monophosphate (cAMP) are also described.

TRIAZOLE DERIVATIVES AND THEIR USE AS PDE4 ACTIVATORS

Field of the Invention

The present invention relates to compounds of Formula I or Formula II, which are activators of long form cyclic nucleotide phosphodiesterase-4 (PDE4) enzymes (isoforms) and to

5   therapies using these activators. In particular, the invention relates to these activator compounds for use in a method for the treatment or prevention of disorders requiring a reduction of second messenger responses mediated by cyclic 3′,5′-adenosine monophosphate (cAMP).

Background to the invention

10   Cyclic 3′,5′-adenosine monophosphate - "cAMP" - is a critical intracellular biochemical messenger that is involved in the transduction of the cellular effects of a variety of hormones, neurotransmitters, and other extracellular biological factors in most animal and human cells. The intracellular concentration of cAMP is controlled by the relative balance between its rate of production and degradation. cAMP is generated by biosynthetic enzymes of the adenylyl

15   cyclase superfamily and degraded by members of the cyclic nucleotide phosphodiesterase (PDE) superfamily. Certain members of the PDE superfamily, such as PDE4, specifically degrade cAMP, while others either specifically degrade cyclic guanosine monophosphate (cGMP) or degrade both cAMP and cGMP. PDE4 enzymes inactivate cAMP, thereby terminating its signalling, by hydrolysing cAMP to 5′-AMP (Lugnier, C. *Pharmacol Ther.* 109:

20   366-398, 2006).

Four *PDE4* genes (*PDE4A*, *PDE4B*, *PDE4C* and *PDE4D*) have been identified, each of which encodes a number of different enzyme isoforms through the use of alternative promoters and mRNA splicing. On the basis of their primary structures, the catalytically active PDE4 splice variants can be classified as "long", "short" or "super-short" forms

25   (Houslay, M.D. *Prog Nucleic Acid Res Mol Biol.* 69: 249-315, 2001). A "dead short" form also exists, which is not catalytically active (Houslay, M.D., Baillie, G.S. and Maurice, D.H. *Circ*

Res. 100: 950-66, 2007). PDE4 long forms have two regulatory regions, called upstream conserved regions 1 and 2 (UCR1 and UCR2), located between their isoform-specific N-terminal portion and the catalytic domain. The UCR1 domain is absent in short forms, whereas the super-short forms not only lack UCR1, but also have a truncated UCR2 domain (Houslay, M.D., Schafer, P. and Zhang, K. *Drug Discovery Today* 10: 1503-1519, 2005).

PDE4 long forms, but not short forms, associate into dimers within cells (Richter, W and Conti, M. *J. Biol. Chem.* 277: 40212-40221, 2002; Bolger, G. B. *et al.*, *Cell. Signal.* 27: 756-769, 2015). A proposed negative allosteric modulation of PDE4 long forms by small molecules has been reported (Burgin A. B. *et al.*, *Nat. Biotechnol.* 28: 63-70, 2010; Gurney M. E. *et al.*, *Handb. Exp. Pharmacol.* 204: 167-192, 2011).

It is known in the art that PDE4 long forms may be activated by endogenous cellular mechanisms, such as phosphorylation (MacKenzie, S. J. et al., *Br. J. Pharmacol.* 136: 421–433, 2002) and phosphatidic acid (Grange et al., *J. Biol. Chem.* 275: 33379-33387, 2000). Activation of PDE4 long forms by ectopic expression of a 57 amino acid protein (called 'UCR1C') whose precise sequence reflects part of that of the upstream conserved region 1 of PDE4D ('UCR1C' sequence reflects that of amino acids 80-136 while UCR is amino acids 17-136: numbering based on the PDE4D3 long isoform) has recently been reported (Wang, L. *et al.*, *Cell. Signal.* 27: 908-922, 2015: "UCR1C is a novel activator of phosphodiesterase 4 (PDE4) long isoforms and attenuates cardiomyocyte hypertrophy"). The authors hypothesised that PDE4 activation might be used as a potential therapeutic strategy for preventing cardiac hypertrophy.

Small molecules that act as activators of PDE4 long forms have not previously been disclosed. Small molecule activators would be desirable for a number of reasons, including ease of manufacture and formulation and improved pharmacokinetic properties.

It is amongst the objects of the present invention to provide small molecule activators of at least one of the long forms of PDE4 of Formula I or Formula II for use in a method of therapy, as well as specific disease treatment or prevention.

Summary of the invention

5   In a first aspect of the invention, there is provided a compound of Formula 1:
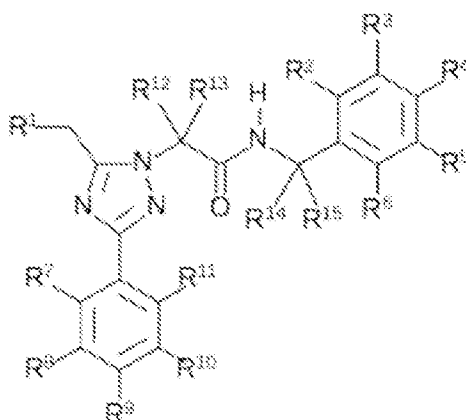
Formula 1

Wherein

R^1 is H, (C1-4) alkyl or (C1-4)alkyloxy, the (C1-4)alkyl and (C1-4)alkyloxy groups being

10   optionally substituted with 1 to 3 fluoros;

R^2 and R^6 are independently selected from H, (C1-4)alkyl, (C1-4)alkyloxy, -CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^3, R^4 and R^5 are independently selected from H, (C1-4)alkyl, (C1-4)alkyloxy, (C1-4)alkylsulfonyl, C(O)-NR^{16}R^{17}, C(O)-OR^{16}, S(O)$_2$-NR^{16}R^{17}, -CN and halogen, the (C1-4)alkyl

15   and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^7, R^8, R^{10} and R^{11} are independently selected from H and F;

R^9 is selected from H, (C1-4)alkyl, (C1-4)alkyloxy, (C1-4)alkylsulfonyl, C(O)-NR^{16}R^{17}, C(O)-OR^{16}, S(O)$_2$-NR^{16}R^{17}, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^{12}, R^{13}, R^{14} and R^{15} are independently selected from H and (C1-4)alkyl;

5   each R^{16} and R^{17}, when present, is independently selected from H and (C1-4)alkyl;

or a pharmaceutically acceptable salt thereof.

The triazole derivative compounds of Formula 1 are shown in the Examples to activate PDE4 long form enzymes, and to provide therapeutically useful effects on cells.

In one embodiment, the present invention provides a compound of Formula 1 for use in

10   therapy. In another embodiment, the therapy is the treatment or prevention of a disease or disorder mediated by excessive intracellular cAMP signalling. In these diseases, a reduction of second messenger responses mediated by cyclic 3',5'-adenosine monophosphate (cAMP) should provide a therapeutic benefit. Also provided is a method of treating or preventing a disease or disorder mediated by excessive intracellular cAMP signalling, comprising the step

15   of administering an effective amount of a compound of Formula 1 to a patient in need thereof. Also provided is the use of a compound of Formula 1 in the manufacture of a medicament for treating or preventing a disease or disorder mediated by excessive intracellular cAMP signalling.

The invention also provides a compound of Formula 2, for use in therapy:
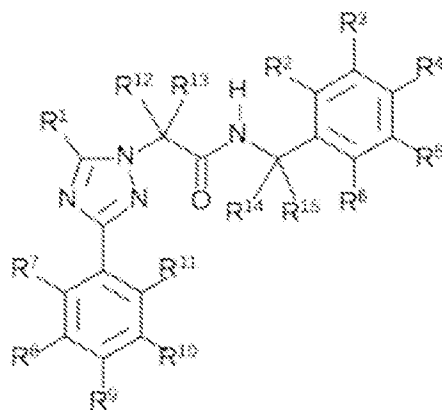
Formula 2

wherein

R^1 is H, (C1-6)alkyl or (C3-7)cycloalkyl, the (C1-6)alkyl and (C3-7)cycloalkyl groups being

5   optionally substituted with 1 to 3 substituents selected from OH, (C1-4)alkyloxy, (C1-4)alkyl-

sulfonyl, $C(O)-NR^{16}R^{17}$, $C(O)-OR^{16}$, $S(O)_2-NR^{16}R^{17}$, CN and halogen;

R^2 and R^6 are independently selected from H, (C1-4)alkyl, (C1-4)alkyloxy, -CN and halogen,

the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^3, R^4 and R^5 are independently selected from H, (C1-4)alkyl, (C1-4)alkyloxy, (C1-

10   4)alkylsulfonyl, $C(O)-NR^{16}R^{17}$, $C(O)-OR^{16}$, $S(O)_2-NR^{16}R^{17}$, -CN and halogen, the (C1-4)alkyl

and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^7, R^8, R^{10} and R^{11} are independently selected from H and F;

R^9 is selected from H, (C1-4)alkyl, (C1-4)alkyloxy, (C1-4)alkylsulfonyl, $C(O)-NR^{16}R^{17}$, $C(O)-$

OR^{16}, $S(O)_2-NR^{16}R^{17}$, -CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being

15   optionally substituted with 1 to 3 fluoros;

R^{12}, R^{13}, R^{14} and R^{15} are independently selected from H and (C1-4)alkyl;

each R^{16} and R^{17}, when present, is independently selected from H and (C1-4)alkyl;

or a pharmaceutically acceptable salt thereof.

Compounds of Formula 2 are shown in the Examples to activate a long form cyclic phosphodiesterase-4 (PDE4) enzyme and to provide therapeutically useful effects on cells.

A compound of Formula 1 or Formula 2 can be provided in a pharmaceutical composition comprising a pharmaceutically acceptable excipient.

5   In certain embodiments of the foregoing aspects, the compounds of the invention are provided for the treatment or prevention of a condition selected from hyperthyroidism, Jansens's metaphyseal chondrodysplasia, hyperparathyroidism, familial male-limited precocious puberty, pituitary adenomas, Cushing's disease, polycystic kidney disease, polycystic liver disease, McCune-Albright syndrome, cholera, whooping cough, anthrax,

10   tuberculosis, HIV, AIDS, Common Variable Immunodeficiency (CVID), melanoma, pancreatic cancer, leukaemia, prostate cancer, adrenocortical tumours, testicular cancer, primary pigmented nodular adrenocortical diseases (PPNAD), Carney Complex, autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD), maturity onset diabetes of young type 5 (MODY5), or cardiac

15   hypertrophy.

In a further aspect, the invention provides a method of preparing a compound of Formula 1 or a pharmaceutically acceptable salt thereof, comprising the step of reacting a compound of Formula 1A
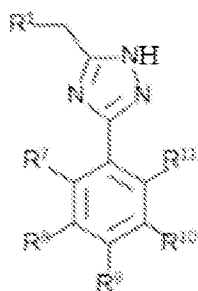
Formula 1A

20   with a compound of Formula 1B
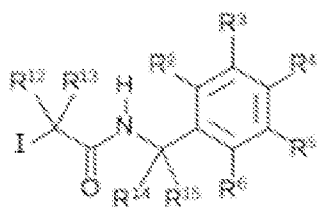
Formula 1B,

wherein R1-R15 are defined for Formula 1 above.

The invention also provides a method of preparing a compound of Formula 1 or a pharmaceutically acceptable salt thereof, comprising the step of reacting a compound of

5   Formula 1C
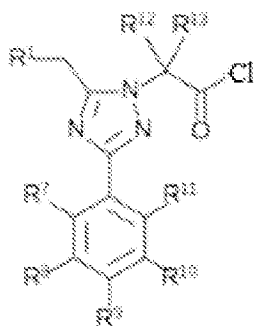
Formula 1C

with a compound of Formula 1D
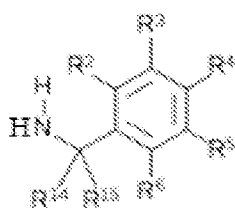
Formula 1D,

wherein R1-R15 are defined for Formula 1 above.

10   Intermediates of Formula 1A and 1C are also provided by the invention.

In yet another aspect, the invention provides a method of identifying a compound able to activate a long form PDE4 enzyme, comprising the steps of:

a. contacting a long form PDE4 enzyme with a candidate compound;

b. determining whether the candidate compound activates the enzyme to at least the same level as a compound of Formula 1 or Formula 2.

The method may be performed according to one of the methods in the Examples. In one

5   embodiment, the method further comprises the prior or subsequent step of determining whether the candidate compound activates a short or super-short PDE4 enzyme. A compound that activates a long form PDE4 and does not activate a short or super-short form PDE4 is selective for long form PDE4. One or more of the compounds exemplified herein may be used as the reference or control.

10   **Brief description of the Figures**

Figure 1 shows activation of PDE4D5, a long form of PDE4, by Examples A, B, D and L;

Figure 2 shows activation of PDE4A4, another long form of PDE4, by Example A;

Figure 3 shows activation of PDE4B1, another long form of PDE4, by Example A;

Figure 4 shows a lack of activation of PDE4B2, a short form of PDE4, by Examples A, B, D

15   and L using the method of Experiment 1, demonstrating selectivity for activation of PDE4 long forms over this PDE4 short form;

Figure 5 shows a reduction in intracellular cAMP levels in HEK293 cells treated with a PDE4 long form activator (10 µM) - Example A - for 10 min prior to forskolin (F) (10 µM) for 2 min;

Figure 6 shows a reduction in intracellular cAMP levels in MDCK cells treated with PDE4

20   long form activators (10 µM) - Examples A, C and D - for 10 min prior to forskolin (F) (10 µM) for 2 min;

Figure 7 shows inhibition of *in vitro* cyst formation in MDCK cells treated with a PDE4 long form activator - Example A - using the method described in Experiment 3, with addition of the specified concentrations of the test compounds every 2 days from day 0 to day 20;

Figure 8 shows a reversal of *in vitro* cyst formation in MDCK cells treated with a PDE4 long form activator - Example A - using the method described in Experiment 4, with addition of the specified concentrations of the test compounds every 2 days from day 10 to day 20;

Figure 9 shows (normalised cell index at 72 hours) that a PDE4 long form activator -

5   Example A - inhibits the proliferation of androgen-sensitive (AS) LNCaP human prostate cancer cells in a concentration dependent manner, using the method described in Experiment 5;

Figure 10 shows (normalised cell index at 72 hours) that, using the method described in Experiment 5, a PDE4 long form activator - Example A - inhibits the proliferation of

10   androgen-insensitive (AI) LNCaP human prostate cancer cells in a concentration dependent manner;

Figure 11 shows the mouse pharmacokinetic profile of Example A, determined by whole blood analysis at defined time points after i.v. and p.o. administration to male C57 mice; and

Figure 12 shows the rat pharmacokinetic profile of Example A, determined by blood plasma

15   analysis at defined time points after i.v. and p.o. administration to male SD rats.

Figure 13 shows (A) concentration dependent inhibition and (B) concentration dependent reversal of *in vitro* cyst formation in a human patient-derived conditionally immortalised OX161 cell line by Example A.

20   **Detailed description of the Invention**

The invention is based on the surprising identification of new compounds that are able to activate long isoforms of PDE4 enzymes. The compounds are small molecules and so are expected to be easier and cheaper to make and formulate into pharmaceuticals than large

biological molecules such as polypeptides, proteins or antibodies. The compounds can be chemically synthesized, as demonstrated in the Examples.

The Examples demonstrate that a number of compounds of Formula 1 and Formula 2 are able to activate long isoforms of PDE4. The Examples go on to demonstrate that certain

5   tested compounds of the invention: do not activate a short form of PDE4, (thereby demonstrating selectivity for activation of PDE4 long forms over PDE4 short forms); reduce intracellular cAMP levels in human and dog cells; inhibit and even reverse *in vitro* cyst formation in human and dog cells; inhibit the proliferation of human prostate cancer cells; and have favourable pharmacokinetic properties. The compounds of the invention are

10   therefore surprisingly advantageous.

Various embodiments of the invention are described herein. It will be recognised that features specified in each embodiment may be combined with other specified features to provide further embodiments.

Compounds – Formula 1

15   A first aspect provides a compound of Formula 1, as set out above.

In an embodiment of the compound of Formula 1, R^7 and R^{11} are H.

In an embodiment of the compound of Formula 1, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are H.

In an embodiment of the compound of Formula 1, R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally

20   substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 1, R^7, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are H and R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 1, R^1 is H, methyl, or methoxy.

In an embodiment of the compound of Formula 1, R^1 is methyl.

In an embodiment of the compound of Formula 1, R^2 and R^6 are each independently selected from H and halogen.

In an embodiment of the compound of Formula 1, R^2 and R^6 are each independently selected from H and fluoro.

In an embodiment of the compound of Formula 1, R^2 and R^6 are each H.

In an embodiment of the compound of Formula 1, R^3, R^4 and R^5 are each independently selected from H, halogen, CN, (C1-4)alkyl and (C1-4)alkyloxy, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 1, R^3 and R^5 are each independently selected from H, fluoro, chloro, methoxy, CN, trifluoromethyl, methoxy and trifluoromethoxy.

In an embodiment of the compound of Formula 1, R^4 is selected from H, fluoro and methoxy.

In an embodiment of the compound of Formula 1, R^9 is selected from methyl, chloro and trifluoromethoxy.

In an embodiment of the compound of Formula 1, R^9 is chloro.

In an embodiment of the compound of Formula 1, one of R^8 and R^{10} is H and the other is fluoro.

In an embodiment of the compound of Formula 1, R^8 and R^{10} are both H.

In an embodiment of the compound of Formula 1, R^9 is chloro, one of R^8 and R^{10} is H and the other of R^8 and R^{10} is fluoro.

In an embodiment, the compound is selected from:

N-(3-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide;

N-Benzyl-2-[3-(4-chlorophenyl)-5-methoxymethyl-1H-1,2,4-triazol-1-yl]acetamide;

N-Benzyl-2-[3-(4-chlorophenyl)-5-methyl-1H-1,2,4-triazol-1-yl]acetamide;

N-(3-Fluorobenzyl)-2-{3-[4-(trifluoromethoxy)-phenyl]-5-methoxymethyl-1H-1,2,4-triazol-1-yl}acetamide;

5   N-(3-Chlorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide;

N-(3-Cyanobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide;

N-[3-(Trifluoromethyl)benzyl]-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide;

N-(3-Methoxybenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide;

10   N-[3-(Trifluoromethoxy)benzyl]-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide;

N-(2-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide;

N-(4-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide;

N-(3,4-Dimethoxybenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-

15   yl]acetamide; and pharmaceutically acceptable salts thereof.

Compounds – Formula 2

A further aspect provides a compound of Formula 2, as set out above.

In an embodiment of the compound of Formula 2, R^7 and R^{11} are H.

In an embodiment of the compound of Formula 2, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are H.

20   In an embodiment of the compound of Formula 2, R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 2, R^7, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are H and R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 2, R^1 is H, methyl, or methoxy.

5   In an embodiment of the compound of Formula 2, R^1 is methyl.

In an embodiment of the compound of Formula 2, R^2 and R^6 are each independently selected from H and halogen.

In an embodiment of the compound of Formula 2, R^2 and R^6 are each independently selected from H and fluoro.

10   In an embodiment of the compound of Formula 2, R^2 and R^6 are each H.

In an embodiment of the compound of Formula 2, R^3, R^4 and R^5 are each independently selected from H, halogen, CN, (C1-4)alkyl and (C1-4)alkyloxy, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 2, R^3 and R^5 each independently selected

15   from H, fluoro, chloro, methoxy, CN, trifluoromethyl, methoxy and trifluoromethoxy.

In an embodiment of the compound of Formula 2, R^4 is selected from H, fluoro and methoxy.

In an embodiment of the compound of Formula 2, R^9 is selected from methyl, chloro and trifluoromethoxy.

In an embodiment of the compound of Formula 2, R^9 is chloro.

20   In an embodiment of the compound of Formula 2, one of R^8 and R^{10} is H and the other is fluoro.

In an embodiment of the compound of Formula 2, R^8 and R^{10} are both H.

In an embodiment of the compound of Formula 2, R^9 is chloro, one of R^8 and R^{10} is H and the other of R^8 and R^{10} is fluoro.

Intermediates of Formula 1A and 1C

Further aspects provide compounds of Formula 1A and 1C, as set out above.

5   In an embodiment of the compound of Formula 1A, R^7 and R^{11} are H.

In an embodiment of the compound of Formula 1A, R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 1A, R^7 and R^{11} are H and R^9 is selected from

10   (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 1A, R^1 is H, methyl, or methoxy.

In an embodiment of the compound of Formula 1A, R^1 is methyl.

In an embodiment of the compound of Formula 1A, R^9 is selected from methyl, chloro and

15   trifluoromethoxy.

In an embodiment of the compound of Formula 1A, R^9 is chloro.

In an embodiment of the compound of Formula 1A, one of R^8 and R^{10} is H and the other is fluoro.

In an embodiment of the compound of Formula 1A, R^8 and R^{10} are both H.

20   In an embodiment of the compound of Formula 1A, R^9 is chloro, one of R^8 and R^{10} is H and the other of R^8 and R^{10} is fluoro.

In an embodiment of the compound of Formula 1C, R^7 and R^{11} are H.

In an embodiment of the compound of Formula 1C, R^{11}, R^{12} and R^{13} are H.

In an embodiment of the compound of Formula 1C, R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

5   In an embodiment of the compound of Formula 1C, R^7, R^{11}, R^{12} and R^{13} are H and R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

In an embodiment of the compound of Formula 1C, R^1 is H, methyl, or methoxy.

In an embodiment of the compound of Formula 1C, R^1 is methyl.

10   In an embodiment of the compound of Formula 1C, R^9 is selected from methyl, chloro and trifluoromethoxy.

In an embodiment of the compound of Formula 1C, R^9 is chloro.

In an embodiment of the compound of Formula 1C, one of R^8 and R^{10} is H and the other is fluoro.

15   In an embodiment of the compound of Formula 1C, R^8 and R^{10} are both H.

In an embodiment of the compound of Formula 1C, R^9 is chloro, one of R^8 and R^{10} is H and the other of R^8 and R^{10} is fluoro.

<u>Definitions</u>

The term "(C1-4)alkyl" as used herein means a branched or unbranched alkyl group having

20   1-4 carbon atoms, optionally containing a ring. Examples of (C1-4)alkyl include butyl, isobutyl, cyclobutyl, tertiary butyl, propyl, isopropyl, cyclopropyl, ethyl and methyl. Where specified in the formulae above, (C1-4)alkyl may be substituted, for example with 1 to 3

fluoros. A particularly preferred example of a substituted (C1-4)alkyl is trifluoromethyl. Alternatively (C1-4)alkyl may be unsubstituted.

The term "(C1-4)alkyloxy" means -O-(C1-4)alkyl wherein (C1-4)alkyl has the meaning as defined above. Examples of (C1-4)alkyloxy include methoxy, ethoxy, propoxy, isopropoxy,

5   butyoxy, isobutoxy and tertiary butoxy. Where specified in the formulae above, (C1-4)alkyloxy may be substituted, for example with 1 to 3 fluoros. A particularly preferred example of a substituted (C1-4)alkyloxy is trifluoromethoxy. Alternatively, (C1-4)alkyloxy may be unsubstituted. In the present invention, alkyloxy is attached to the rest of the molecule by the "oxy" moiety.

10   The term "halogen" means F, Cl, Br or I. F and Cl are particularly preferred.

The 1,2,4-triazole derivatives of Formula 1 or 2 may be prepared by methods known in the art of organic chemistry in general. Suitable methods for constructing 1,2,4-triazole rings are, for example, described in the general reference Katritzky, A.R.: Comprehensive heterocyclic chemistry (First Edition, Pergamon Press, 1984, see especially Volume 5, Part 4A, Five-

15   membered rings with two or more nitrogen atoms). Suitable protecting groups for functional groups which are to be temporarily protected during syntheses are known in the art, for example from Wuts, P.G.M. and Greene, T.W.: Protective Groups in Organic Synthesis, Fourth Edition, Wiley, New York, 2006.

Activation of long PDE4 Isoforms

20   PDE4 long isoforms have two regulatory regions, upstream conserved region 1 (UCR1) and upstream conserved region 2 (UCR2). These are between the isoform-specific N-terminal portion and the catalytic domain. The UCR1 domain is missing in the short forms, whereas the super-short forms not only lack UCR1, but also have a N-terminal truncated UCR2 domain (Houslay, M. D., Schafer, P. and Zhang, K. Drug Discovery Today 10: 1503-1519,

25   2005).

There are four PDE4 families, PDE4A, PDE4B, PDE4C and PDE4D. The present invention concerns compounds that are capable of activating one or more of the long isoforms from one or more of these four families. The long isoform PDE4 may therefore be long isoform PDE4A, long isoform PDE4B, long isoform PDE4C or long isoform PDE4D. For the avoidance of doubt, a long isoform PDE4 comprises a UCR1 region. Typically, the long isoform PDE4 is human. UCR1 is conserved within mammalian species (Houslay, MD, Sullivan, M and Bolger GB Adv Pharmacol. 1998;44:225-34), so in other embodiments, the long isoform PDE4 can be from a non-human mammal.

Without wishing to be bound by theory, PDE4 long form activators of Formula I or Formula II of the present invention are small molecules that are believed to bind directly to PDE4 long forms and induce structural changes that increase, stabilise, uncover and/or maintain the catalytic activity of these enzymes. In the field of pharmacology, and as used herein, a small molecule is defined as a low molecular weight organic compound that may serve as a regulator of biological processes. A small molecule activator according to the present invention has a molecular weight of less than or equal to 700 Daltons. This allows for the possibility to rapidly diffuse across cell membranes and reach intracellular sites of action (Veber, D. F. et al., *J. Med. Chem.* 45: 2615–2623, 2002). The preferred molecular weight for a small molecule activator according to the present invention is greater than or equal to 200 Daltons and less than or equal to 600 Daltons. Especially preferred small molecule activators according to the present invention have molecular weights of greater than or equal to 250 Daltons and less than or equal to 500 Daltons (Lipinski, C. A. *Drug Discovery Today: Technologies* 1: 337–341, 2004).

One suitable method of detecting whether or not a compound is capable of serving as an activator of a PDE4 long form is using a two-step radio-assay procedure described in Experiment 1. In summary, the method involves incubating a PDE4 long form with a test small molecule activator, together with [^{3}H]-labelled cAMP to assess alterations in the breakdown of cAMP to the 5'- adenosine monophosphate (5'-AMP) product. A sample of

the reaction mixture from such an incubation is subsequently treated with snake venom 5'-nucleotidase to allow conversion of the nucleotide [^{3}H]-labelled 5'-AMP to the uncharged nucleoside [^{3}H]-labelled adenosine, which can be separated and quantified to assess PDE4 activity and the effect of the test compound (Thompson, W. J. and Appleman, M. M.

5   *Biochemistry* 10: 311-316, 1971, with some modifications as described in: Marchmont, R. J. and Houslay, M. D. *Biochem J.* 187: 381-92, 1980).

Using the above assay procedure, as described in detail in Experiment 1, preferred small molecule activators according to the present invention produce an increase in the background activity of one or more PDE4 long forms of <u>more than 50%</u> at a test compound

10   concentration of 100 micromolar or less. Especially preferred small molecule activators according to the present invention produce an increase in the background activity of one or more PDE4 long forms of more than 50% at a test compound concentration of 10 micromolar, or less, for example 3 micromolar.

The compounds of the present invention may be selective for the long form of the PDE4

15   enzyme and, as such, do not act or act to a lesser extent as activators of the short or super-short isoforms of the PDE4 enzyme. The short or super-short isoform PDE4 may therefore be short or super-short isoform PDE4A, short or super-short isoform PDE4B, short or super-short isoform PDE4C, or short or super-short isoform PDE4D. For the avoidance of doubt, short and super-short isoforms of PDE4 lack a UCR1 domain. Super-short isoforms

20   comprise a truncated UCR2 domain. Typically, the short or super-short isoform PDE4 is human, but may also be from other mammalian species (where UCR2 is conserved, see Houslay, MD, Sullivan, M and Bolger GB Adv Pharmacol. 1998;44:225-34).

Under the same assay conditions, as described in Experiment 1, the small molecule activators according to the present invention typically produce a less than 50% increase in

25   the background activity of the short or super-short forms of the PDE4A, PDE4B, PDE4C or PDE4D enzymes at a test compound concentration of 100 micromolar, or less.

Typical compounds may therefore provide a positive result in an assay for activation of a long form PDE4 and a negative result in an assay for activation of a short form (or super-short form) of PDE4. In one embodiment, the compound activates long isoform PDE4A and does not activate either of short and super-short isoform PDE4A. In another embodiment,

5   the compound activates long isoform PDE4B and does not activate either of short and super-short isoform PDE4B. In a further embodiment, the compound activates long isoform PDE4C and does not activate either of short and super-short isoform PDE4C. In another embodiment, the compound activates long isoform PDE4D and does not activate either of short and super-short isoform PDE4D.

10   PDE4 long isoforms include those now known as PDE4A4, PDE4A4/5, PDE4A5, PDE4A8, PDE4A10 , PDE4A11, PDE4B1, PDE4B3, PDE4B4, PDE4C1, PDE4C2, PDE4C3, PDE4D3, PDE4D4, PDE4D5, PDE4D7, PDE4D8, PDE4D9 and PDE4D11. Further long isoforms may be or have already been identified or called by different nomenclature from any of the four PDE4 sub-families.

15   PDE4 short isoforms include PDE4A1, PDE4B2, PDE4D1 and PDE4D2. Further short isoforms may be or have already been identified or called by different nomenclature from any of the four PDE4 sub-families.

PDE4 super-short isoforms include PDE4B5, PDE4D6 and PDE4D10. Further super-short isoforms may be or have already been identified or called by different nomenclature from

20   any of the four PDE4 sub-families.

The Examples below exemplify activity of compounds in human PDE4D5, PDE4A4 and PDE4B1 long isoforms and a lack of activity in the human PDE4B2 short isoform. Details of these isoforms and a number of the other known isoforms, including GenBank accession numbers, are provided in Tables A to D immediately below.

PDE4A				
Isoform	**accession**	**calculated size**	**SDS-PAGE (kDa)**	**Type**
A1 (human)	U97583		83	S
A1 (rodent)	M26715, L27062		76	S
A4* (humanA5)	L20965		125	L
A5 (rodentA4)	L27057		107	L
A7** (human)	U18088		37	DS
A8 (rodent)	L36467	88 kDa	98	L
A10 (human)	AF110461	91 kDa	121	L
A11 (human)	AY618547	95 kDa	126	L

L = Long; S = short; SS = Super-short; D = Dead short

* Note that the PDE4A4B clone is correct while PDE4A4A has a cloning artefact and PDE4A4C is a truncation artefact.

** Note that this species is C- as well as N-terminally truncated and so will NOT be detected by pan PDE4A antisera that detect all active forms.

Table A – Examples of known PDE4A isoforms

PDE4B			
Isoform	**accession**	**SDS-PAGE (kDa)**	**Type**
B1	L20966	104	L
B2	M97515, L20971	68	S
B3	U85048	103	L
B4	AF202733	84	L
B5	EF595686	58	SS
L = Long; S = short; SS = Super-short; D = Dead short			

Table B – Examples of known PDE4B Isoforms

PDE4C		
Isoform Name	**GenBank**	**Size (aa)**
PDE4C1 (partial clone)	L20968	251*(partial)
PDE4C1	Z46632	712* (Long)
PDE4C2	U88712	606 (Long)
PDE4C3	U88713	700* (Long)
PDE4C4	U66346	791* (Long)
PDE4C5	U66347	426*
PDE4C6	U66348	518*
PDE4C7	U66349	427*

5   Table C – Examples of known PDE4C Isoforms

PDE4D

Isoform	accession	calculated	SDS-PAGE (kDa)	Type
D1	U50157, U79571	66 kDa	68	S
D2	U50158, AFO12074	66 kDa	68	S
D3	L20970, U50159	77 kDa	95	L
D4	L20969	91 kDa	119	L
D5	AFO12073	84 kDa	105	L
D6 (m)	AF536975	59 kDa	59	SS
D7	AF536976	85 kDa	103	L
D8*	AF536977	78 kDa	96	L
D9	AY245867	77 kDa	95	L
D10	DQ665896	58 kDa	58	SS
D11	EU489880	79 kDa	95	L

L = Long; S = short; SS = Super-short; D = Dead short

* nb D8 was originally called PDE4D6 in the literature

(m) Memory clones are (AY245867, AF536977, AF536976)

Table D – Examples of known PDE4D Isoforms

Reduction of cAMP

Without wishing to be bound by theory, the compounds of the present invention may function by reducing cAMP levels in one or more intracellular compartments. The PDE4 long form activators of the present invention may thus provide a means to regulate certain cellular

5   processes that are dependent upon cAMP.   Excessive intracellular cAMP signalling mediates a number of diseases and disorders.   Therefore, the compounds of the invention are expected to be of utility for the treatment of diseases associated with abnormally elevated cAMP levels, increased cAMP-mediated signalling and/or reduced cAMP elimination, enzymatic or otherwise (e.g. via efflux). The treatment is typically of a human,

10   but may also be of a non-human animal, such as a non-human mammal (e.g. veterinary treatment).

In one aspect, the present invention provides a small molecule activator of a PDE4 long form of Formula 1 or Formula 2, for use in a method for the treatment or prevention of disorders where a reduction of second messenger responses mediated by cyclic 3',5'-adenosine

15   monophosphate (cAMP) is required.

For example, gain-of-function gene mutations in proteins involved in driving cAMP signalling upstream of adenylyl cyclase, such as GPCRs and Gsα, can lead to abnormal excessive cAMP activity with pathological consequences (Lania A, Mantovani G, Spada A. *Ann Endocrinol (Paris)*. 73: 73-75, 2012.; Thompson, M. D. *et al., Methods Mol. Biol.* 448: 109-

20   137, 2008; Weinstein LS, Liu J, Sakamoto A, Xie T, Chen M. *Endocrinology*. 145: 5459-5464, 2004; Lania A, Mantovani G, Spada A. *Eur J Endocrinol*. 145: 543-559, 2001). PDE4 long form activators of the present invention, possessing the ability to accelerate the termination of cAMP action, would therefore be expected to be effective in the treatment, prevention or partial control of diseases characterised by undesirably high cAMP levels, or

25   activity, as detailed below.

<u>Diseases characterised by elevated cAMP levels</u>

Hyperthyroidism

Stimulation of the thyroid-stimulating hormone (TSH) receptor (TSHR) leads to increased

5   generation and release of thyroid hormones, thyroxine and triiodothyronine, through a cAMP-dependent signalling mechanism involving Gsα-mediated activation of adenylyl cyclase. Gain-of-function mutations in the TSHR have been reported to be involved in the development of hyperthyroidism (Duprez, L. *et al.*, *Nat. Genet.* 7: 396-401, 1994; Biebermann, H. *et al.*, *J. Clin. Endocrinol. Metab.* 86: 4429-4433, 2001; Karges, B. *et al.*, *J.*

10   *Endocrinol.* 186: 377-385, 2005). Activating mutations of both TSHR and Gsα have also been found in goitre and thyroid adenomas (Arturi, F. et al., *Exp. Clin. Endocrinol. Diabetes* 106: 234-236, 1998). The increased cAMP activity in thyroid adenomas, as a result of the activating TSHR or Gsα mutations, has been reported to produce a protective adaptive increase in PDE4 activity to counteract abnormal rise in cAMP levels and signal transduction

15   (Persani, L. *et al.*, *J. Clin. Endocrinol. Metab.* 85: 2872-2878, 2000).

The most common cause of hyperthyroidism is Graves' disease, an autoimmune disorder in which antibodies mimic TSH action at the TSHR, leading to excessive cAMP activity in thyroid follicle cells and consequently a state of hyperthyroidism.

PDE4 long form activators of the present invention are therefore expected to be effective in

20   the treatment, prevention or partial control of hyperthyroidism.   In one embodiment, the hyperthyroidism is associated with Graves' disease.

Jansens's Metaphyseal Chondrodysplasia and Hyperparathyroidism

Jansens's Metaphyseal Chondrodysplasia (JMC) is a very rare disease resulting from gain-of-function mutations of the parathyroid hormone (PTH) receptor 1 (PTHR1) (Thompson, M.

25   D. *et al.*, *Methods Mol. Biol.* 448: 109-137, 2008). The constitutive activation of the PTHR1

which couples to adenylyl cyclase as effector is associated with excessive cAMP signalling primarily in bone and kidney, leading to dysregulation of ion homeostasis characterised by hypercalcemia and hypophosphatemia (Calvi, L.M. and Schipani, E. *J. Endocrinol. Invest.* 23: 545-554, 2000) and developmental (e.g. short stature) and physical (e.g. protruding eyes) abnormalities.

Primary hyperparathyroidism results from excessive release of PTH from the parathyroid gland due to tissue enlargement or non-cancerous adenoma. The resulting excessive stimulation of the PTHR1 receptor causes disruption of plasma ion homeostasis with patients showing hypercalcemia and hypophosphatemia. PDE4 long form activators of the present invention are therefore expected to be effective in the treatment, prevention or partial control of JMC and hyperparathyroidism.

Familial Male Precocious Puberty (Testotoxicosis)

Familial male-limited precocious puberty (FMPP), also known as familial sexual precocity or gonadotropin-independent testotoxicosis, is a disorder in which boys generally develop signs of precocious puberty in early childhood.

The spinal length in boys may be short due to a rapid advance in epiphyseal maturation. FMPP is an autosomal dominant condition with constitutively activating mutations in the luteinizing hormone (LH) receptor, which leads to increased cAMP production, associated with Leydig cell hyperplasia and low sperm cell count (Latronico, A.C. *et al.*, *J Clin. Endocrinol. Metab.* 80: 2490-2494, 1995; Kosugi, S. *et al.*, *Hum. Mol. Genet.* 4: 183-188, 1995). PDE4 long form activators of the present invention are therefore expected to be effective in the treatment, prevention or partial control of FMPP.

Pituitary Adenomas and Cushing's Disease

Non-cancerous tumours of the pituitary gland are collectively referred to as pituitary adenomas and can lead to hypersecretion of adenohypophyseal hormones (e.g. growth

hormone, thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone and adrenocorticotrophic hormone), which exert their action through GPCRs coupled to Gs and cAMP generation. Thus pituitary adenomas can lead to a state of enhanced cAMP mediated signalling in a variety of endocrine tissues which can precipitate a number of hormonal

5   disorders such as acromegly (mainly due to excess growth hormone secretion), Cushing's disease (due to overproduction of adrenocorticotrophic hormone (ACTH) and the subsequent hypercortisolemia) and/or general hyperpituitarism (associated with excess release of multiple anterior pituitary hormones). Current treatment options for pituitary adenomas include treatment with dopamine receptor agonists, which reduce tumour size

10   and lower pituitary hormonal output through a mechanism involving lowering of intracellular cAMP levels. PDE4 long form activators of the present invention may also be expected to attenuate the pathological effects of pituitary hormones in their target tissues, such as the adrenal glands.

In Cushing's disease, pituitary adenoma related overproduction of ACTH can lead to

15   hypercortisolemia through an overactivation of melanocortin 2 receptor (MC2) and subsequent cAMP mediated stimulation of steroidogenesis and release of cortisol from the adrenal cortex (Tritos, N. A. and Biller, B. M. *Discov. Med.* 13: 171-179, 2012). PDE4 long form activators of the present invention are therefore expected to be effective in the treatment, prevention or partial control of Cushing's disease.

20   *Polycystic kidney disease*

Polycystic kidney disease (PKD) is a genetic disorder of the kidneys characterised by development of pathological cysts, which damage renal structure and compromise kidney function (Takiar, V. and Caplan, M. J. *Biochim. Biophys. Acta.* 1812: 1337-1343, 2011; Masoumi, A. *et al.*, *Drugs* 67: 2495-2510, 2007). There are two types of PKD: autosomal

25   dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). ADPKD affects between 0.1% and 0.2% of the population worldwide and

is characterized by progressive cyst development and enlarged kidneys. Approximately 50% of people with this disease will develop end stage kidney disease, usually between 40 and 70 years of age and require dialysis or kidney transplantation. ARPKD affects around 1:20,000 live births and is typically identified in the first few weeks after birth. Pulmonary

5   hypoplasia results in a 30-50% death rate in neonates with ARPKD.

Defects in two genes are thought to be responsible for ADPKD. In around 85% of patients, development of ADPKD can be linked to mutations in the gene PKD1, encoding polycystin-1 (PC-1); in around 15% of patients mutations in PKD2, encoding polycystin-2 (PC-2) are implicated. Cyclic AMP has been identified as an important stimulus for proliferation and cyst

10   expansion in polycystic kidney cells but not in normal human kidney cells (Yamaguchi, T. *et al.*, *Kidney Int.* 57: 1460-1471, 2000). A considerable body of evidence has now developed to implicate cAMP as an important facilitator of renal cystogenesis (Masoumi, A. *et al.*, *Drugs* 67: 2495-2510, 2007; Wallace, D. P. *Biochim. Biophys. Acta.* 1812: 1291-1300, 2011). Consistent with the role of cAMP in cyst formation, agents that lower cAMP levels (e.g.

15   vasopressin V2 receptor antagonists and the somatostatin receptor agonist octreotide) showed efficacy in rodent models of PKD (Torres, V. E. *et al.*, *Nat. Med.* 10: 363-364, 2004; Gattone, V. H. 2nd et al., *Nat. Med.* 9: 1323-1326, 2003; Belibi, F. A. and Edelstein, C. L. *Expert Opin. Investig. Drugs.* 19: 315-328, 2010). In zebrafish embryos, depletion of a cAMP-hydrolysing PDE enzyme subtype, PDE1A, resulted in development of a cystic

20   phenotype, while PDE1A over-expression partially rescued cystic phenotypes resulting from PC2 depletion (Sussman, C. R., Ward, C. J., Leightner, A. C., Smith, J. L., Agarwal, R., Harris, P. C., Torres, V. E. *J. Am. Soc. Nephrol.* 25: 2222-2230, 2014). Phosphodiesterase activation has been suggested as a therapeutic strategy for PKD treatment (Sun, Y., Zhou, H. and Yang, B-X. *Acta Pharmacologica Sinica* 32: 805–816, 2011).

25   PDE4 long form activators of the present invention are therefore expected to be effective in the treatment, prevention or partial control of polycystic kidney disease.

The Examples, in particular Figures 7 and 8, show the inhibition and reversal of *in vitro* cyst formation in dog MDCK cells treated with a PDE4 long form activator - Example A - using the methods described in Experiment 3, with addition of the specified concentrations of the test compounds every 2 days from day 0 to day 20, and Experiment 4, with addition of the specified concentrations of the test compounds every 2 days from day 10 to day 20.

The Examples, in particular Figure 13, show the inhibition and reversal of *in vitro* cyst formation in human OX161 cells treated with a PDE4 long form activator - Example A - using the methods described in Experiment 9, with addition of the specified concentrations of the test compound every 2 days from day 0 to day 10, and Experiment 10, with addition of the specified concentrations of the test compound every 2 days from day 10 to day 20.

This provides experimental confirmation of the rationale that compounds of the invention are able to treat diseases and disorders mediated by cAMP.

Polycystic Liver Disease

Polycystic liver disease (PLD) is a rare inherited condition associated with hepatic cystogenesis (usually defined when number of cysts exceeds 20), which often occurs in association with ADPKD (Strazzabosco, M. and Somlo, S. *Gastroenterology* 140: 1855-1859, 2011; Gevers, T. J. and Drenth, J. P. *Curr. Opin. Gastroenterol.* 27: 294-300, 2010). PLD may have a different genetic pathology when compared to ADPKD, driven by mutated proteins associated with the endoplasmic reticulum and the cilium. Increased cholangiocyte proliferation, neovascularisation and elevated fluid secretion act to drive liver cyst formation through dysregulation of multiple signal transduction pathways, including cAMP-mediated signalling. Elevation of hepatic cAMP levels stimulates cAMP-dependent chloride and fluid secretion in biliary epithelial cells and increases cholangiocyte proliferation (Janssen, M. J. *et al.*, *J. Hepatol.* 52: 432-440, 2010). Somatostatin, which acts through a Gi-coupled mechanism to lower cAMP levels, reduced cholangiocyte proliferation and fluid secretion (Gong, A.Y. *et al.*, *Am. J. Physiol. Cell. Physiol.* 284: C1205-1214, 2003). Furthermore, the

synthetic somatostatin analogue, octreotide, showed efficacy in an animal model of PLD through a mechanism involving reduction in cAMP signalling (Masyuk, T.V. *et al.*, *Gastroenterology* 132: 1104-1116, 2007). PDE4 long form activators of the present invention may therefore be effective in the treatment, prevention or partial control of polycystic liver

5   disease due at least in part to cAMP.

Maturity onset diabetes of young type 5 (MODY5)

MODY5 is a form of non-insulin-dependent diabetes mellitus associated with renal cysts. It is an autosomal dominant disorder caused by mutations in the gene encoding hepatocyte nuclear factor-1β (HNF-1β). The predominant clinical feature of patients affected by MODY5

10   is renal dysfunction, frequently diagnosed before the onset of diabetes. In some patients, HNF-1β mutations can result in additional phenotypic features, such as pancreatic atrophy, abnormal liver function and genital tract abnormalities. Studies in mice suggest that the mechanism responsible for renal cyst formation, associated with mutations of HNF-1β, involves a severe defect of the transcriptional activation of PKD2, in addition to effects on

15   uromodulin (UMOD) and PKD1 genes. Down-regulation of PKD1 and PKD2 is associated with cAMP-driven formation of renal cysts (Mancusi, S. *et al.*, *J. Nephrol.* 26: 207-12, 2013). HNF-1β also binds to the PDE4C promoter and regulates the expression of PDE4C (Ma *et al.*, *PNAS* 104: 20386, 2007).

PDE4 long form activators of the present invention are therefore expected to be effective in

20   the treatment, prevention or partial control of the symptoms of MODY5.

Cardiac hypertrophy, heart failure and arrhythmia

Localized regulation and integration of cAMP signalling are important for proper cardiac function and perturbation of this signalling can lead to heart failure. Upon chronic β-adrenergic receptor stimulation, cardiomyocyte hypertrophy is induced via elevated cAMP

25   and activation of its downstream effectors, including PKA and Epac (Wang, L. *et al.*, *Cell.*

Signal. 27: 908-922, 2015 and references therein). Cardiomyocyte hypertrophy increases the risk of heart failure and arrhythmia.

PDE4 long form activators of the present invention may therefore be effective in the treatment, prevention or partial control of cardiac hypertrophy, heart failure and/or arrhythmia.

Diseases associated with increased cAMP-mediated signalling

Disorders associated with activating mutations of the alpha subunit of the G protein (GNAS1)

The G-protein Gs acts as a transducer for GPCRs that stimulate adenylyl cyclase activity and exert their biological effects by increasing intracellular cAMP levels. Gs is a heterotrimeric protein composed of α, β and γ subunits. Activating mutations in the gene, GNAS1, for the α-subunit have been identified which lead to exaggerated abnormal cAMP signalling in a variety of tissues and give rise to a range of disorders.

McCune-Albright syndrome

McCune-Albright syndrome (MAS) is a rare genetic disorder typically characterised by three dominating features of precocious puberty, fibrous dysplasia of bone and café au lait lesions. The underlying molecular pathology for MAS involves an activating mutation of the GNAS1 gene (Diaz, A. Danon, M. and Crawford, J. *J. Pediatr. Endocrinol. Metab.* 20: 853-880, 2007). PDE4 long form activators of the present invention would therefore be expected to be effective in the treatment, prevention or partial control of disorders associated with activating mutations of GNAS1, including McCune-Albright syndrome.

Amelioration of toxin-induced increases in adenylyl cyclase activity in infectious diseases.

Adenylyl cyclase, the enzyme responsible for production of cAMP, is a key biological target thought to be involved in mediating the effects of many bacterial toxins (Ahuja et al., Critical

Reviews in Microbiology, 30: 187-196, 2004). These toxins produce their effects by raising cAMP levels through enhancement of host immune cell and/or pathogen related adenylyl cyclase activity. PDE4 long form activators of the present invention, by reducing cAMP levels, would therefore be expected to be of utility in the treatment or partial control of

5   symptoms of infectious diseases that are associated with elevated cAMP activity. The following are some examples of such infectious diseases:

Cholera

Vibrio cholerae produces cholera toxin, which through adenosine disphosphate ribosylation of the α subunit of Gs leads to host cell adenylyl cyclase activation and cAMP production.

10   Diarrhoea caused by cholera toxin is believed to be a result of excessive cAMP accumulation in the cells of the gastrointestinal tract.

Whooping Cough

Bordetella pertussis is the pathogen responsible for the childhood disease whooping cough. *Bordetella pertussis* toxin stimulates adenosine disphosphate ribosylation of the α subunit of

15   Gi and indirectly augments cAMP levels in target cells. The bacterium also secretes an invasive adenylyl cyclase, which produces toxic cAMP levels and impairs host immune defence.

Anthrax

Anthrax is caused by *Bacillus anthracis* and whilst it is primarily an animal disease it can be

20   transmitted to humans through contact. Anthrax infections are associated with widespread oedema, the development of which is thought to be driven by oedema toxin. The latter is an adenylyl cyclase and is activated by host calmodulin to produce abnormally high levels of cAMP that have a toxic effect on host immune cells.

Tuberculosis

Mycobactrium tuberculosis expresses a large and diverse range of adenylyl cyclases, which may play a role in virulence and generation of disease pathology. One adenylyl cyclase subtype, RV0386, has been demonstrated to enter host macrophages and elevate intracellular cAMP to cause toxicity (Agarwal et al., *Nature*, 460: 98-102, 2009).

5   PDE4 long form activators of the present invention may therefore be effective in the treatment, prevention or partial control of infectious diseases such as cholera, whooping cough, anthrax and tuberculosis.

Diseases dependent upon activation of PKA by elevated cAMP.

In eukaryotes, cAMP activates protein kinase A (PKA), which is also known as cAMP-
10   dependent protein kinase. PKA is normally inactive as a tetrameric holoenzyme, consisting of two catalytic and two regulatory units, with the regulatory units blocking the catalytic centres of the catalytic units. cAMP binds to specific locations on the regulatory units of PKA and causes dissociation between the regulatory and catalytic units, thus activating the catalytic units. The active catalytic units catalyse the transfer of phosphate from ATP to
15   specific residues of protein substrates, which may modulate the function of those protein substrates.

PDE4 long form activation reduces cAMP levels and cAMP mediated activation of PKA. PDE4 long form activators of the present invention would therefore be expected to be of utility in the treatment or partial control of disorders where inhibitors of PKA show evidence
20   of therapeutic effects.

Disorders that are dependent upon activation of PKA by cAMP may be identified by their response to PKA inhibitors such as Rp-8-Br-cAMPS. Rp-8-Br-cAMPS is an analogue of cAMP that occupies the cAMP binding sites of PKA, preventing its dissociation and activation.

25   *HIV infection and AIDS*

T cells from HIV-infected patients have increased levels of cAMP and are more sensitive to inhibition by Rp-8-Br-cAMPS than are normal T cells. Excessive activation of PKA by cAMP has been associated with the progressive T cell dysfunction in HIV infection (Aandahl, E. M. et al., FASEB J. 12: 855–862, 1998). Furthermore, in vivo administration of Rp-8-Br-cAMPS

5 has been shown to restore T cell responses in retrovirus-infected mice (Nayjib, B. et al., The Open Immunology Journal, 1: 20-24, 2008). PDE4 long form activators of the present invention are therefore expected to be of utility in the treatment, prevention or partial control of HIV infection and AIDS.

Common Variable Immunodeficiency (CVID)

10 In vitro administration of Rp-8-Br-cAMPS has been shown to correct impaired secretion of the cytokine IL-10 by T cells from patients with Common Variable Immunodeficiency (CVID) (Holm, A. M. et al., J. Immunol. 170: 5772-5777, 2003). PDE4 long form activators of the present invention are therefore expected to be of utility in the treatment, prevention or partial control of CVID.

15 <u>Diseases dependent upon activation of either or both of Epac1 and Epac2 by elevated cAMP.</u>

In addition to PKA, cAMP activates another intracellular receptor, known as exchange protein directly activated by cAMP (Epac). There are two isoforms of Epac, Epac1 and Epac2, both consisting of a regulatory region that binds cAMP and a catalytic region that

20 promotes the exchange of GDP for GTP on the small G proteins, Rap1 and Rap2 of the Ras family. In addition, Epac proteins exert their functions through interactions with a number of other cellular partners at specific cellular loci. Pathophysiological changes in Epac signalling have been associated with a wide range of diseases (Breckler, M. et al., Cell. Signal. 23: 1257-1266, 2011).

25 Relevant disorders that are dependent upon activation of Epac proteins by cAMP may be identified by their response to Epac inhibitors, such as ESI-09, a novel non-cyclic nucleotide

Epac1 and Epac2 antagonist that is capable of specifically blocking intracellular Epac-mediated Rap1 activation and Akt phosphorylation, as well as Epac-mediated insulin secretion in pancreatic beta cells (Almahariq, M. *et al.*, *Mol. Pharmacol.* 83: 122-128, 2013).

Melanoma

5   Epac1 has been implicated in promoting migration and metastasis in melanoma (Baljinnyam, E. et al., *Pigment Cell Melanoma Res.* 24: 680-687, 2011 and references cited therein).

PDE4 long form activators of the present invention are therefore expected to be of utility in the treatment, prevention or partial control of melanoma.

Pancreatic cancer

10   It has recently been shown that Epac1 is markedly elevated in human pancreatic cancer cells as compared with normal pancreas or surrounding tissue (Lorenz, R. *et al.*, *Pancreas* 37: 102-103, 2008).

Pancreatic cancer is often resistant to treatments that are usually effective for other types of cancer. Using the Epac inhibitor ESI-09, a functional role of Epac1 overexpression in

15   pancreatic cancer cell migration and invasion was demonstrated (Almahariq, M. *et al.*, *Mol. Pharmacol.* 83: 122-128, 2013). These findings are consistent with results based on RNAi silencing techniques and suggest that inhibition of Epac1 signalling could be an effective therapeutic strategy for pancreatic cancer.

PDE4 long form activators of the present invention would therefore be expected to be of

20   utility in the treatment, prevention or partial control of pancreatic cancer.

Diseases dependent upon modulation of cAMP-gated ion channels by elevated cAMP.

In addition to activation of PKA and Epac, another effector pathway for elevated cAMP is the activation of cAMP-gated ion channels. PDE4 long form activators of the present invention

would therefore be expected to be of utility in the treatment of disorders where inhibitors of cAMP-gated ion channels show evidence of therapeutic effects.

Diseases associated with increased activity of cAMP response element binding protein.

The cAMP response element binding protein (CREB) is an important transcription factor involved in the regulation of a variety of cellular functions such as cell proliferation, differentiation, survival, and apoptosis (Cho et al., *Crit Rev Oncog*, 16: 37-46, 2011). CREB activity is regulated by kinase dependant phosphorylation through a range of extracellular signals, such as stress, growth factors and neurotransmitters. Phosphorylation leads to dimerisation of CREB, and together with other co-activator partner proteins, enables it to bind to promoter regions of target genes containing the cAMP response element (CRE sites) and initiate transcriptional activity. The cAMP pathway (e.g. through cAMP-dependant protein kinase mediated phosphorylation) is an important positive modulator of CREB mediated biological activity. PDE4 long form activators of the present invention are therefore expected to be of utility in the treatment, prevention or partial control of disorders associated with elevated CREB activity.

Leukaemia

Bone marrow cells from acute lymphoid and myeloid leukaemia patients have been reported to overexpress CREB protein and mRNA (Crans-Vargas et al., *Blood*, 99: 2617-9, 2002; Cho et al., *Crit Rev Oncog*, 16: 37-46, 2011). Furthermore, the increased CREB level correlates with poor clinical response in subjects with acute myeloid leukaemia (Crans-Vargas et al., *Blood*, 99: 2617-9, 2002; Shankar et al., *Cancer Cell*, 7:351-62, 2005). Upregulation of CREB is associated with stimulation of human leukaemia cell growth whilst downregulation inhibits myeloid cell proliferation and survival. PDE4 long form activators of the present invention would be expected to reduce CREB activity and function through attenuation of cAMP mediated stimulation of CREB and therefore expected to have utility in the treatment, prevention or partial control of acute lymphoid and myeloid leukaemia.

Prostate Cancer

Abnormal excessive androgen activity is an important driver in the development of prostate cancer as it stimulates the development of intraepithelial neoplasias (Merkle et al., *Cellular Signalling*, 23: 507-515, 2011). This is strongly supported by the use of androgen ablation

5   approaches, involving chemical or surgical castration, in the treatment of prostate cancer. Cyclic AMP elevating agents such as forskolin can enhance androgen receptor activity through multiple intracellular mechanisms including androgen receptor activation through phosphorylation and/or interaction with CREB. Epac1 activation has also been implicated in promoting cellular proliferation in prostate cancer (Misra, U. K. and Pizzo, S. V. *J. Cell.*

10 *Biochem.* 108: 998-1011, 2009; Misra, U. K. and Pizzo, S. V. *J. Cell. Biochem.* 113: 1488-1500, 2012). PDE4 long form activators of the present invention are therefore expected to have utility in the treatment, prevention or partial control of prostate cancer.

The Examples demonstrate that compounds of the invention are able to inhibit the proliferation of prostate cancer cells. This provides experimental confirmation of the

15 rationale that compounds of the invention are able to treat diseases and disorders mediated by cAMP.

Diseases associated with reduced activity of cAMP-hydrolysing PDE enzymes

Loss-of-function mutations in gene(s) for cAMP-hydrolysing PDE isoforms other than PDE4, such as PDE8 and PDE11, have been detected in a number of diseases (Vezzosi, D. and

20 Bertherat, J., *Eur. J. Endocrinol.* 165: 177-188, 2011; Levy, I. *et al.*, *Curr. Opin. Pharmacol.* 11: 689-697, 2011; Azevedo, M. F. and Stratakis, C. A. *Endocr. Pract.* 17 Suppl 3: 2-7, 2011). These mutations can lead to abnormally high cAMP levels and/or duration of cAMP action with pathological consequences as detailed below. PDE4 long form activators of the present invention are therefore expected to be of utility in the treatment, prevention or partial

25 control of these diseases, such as adrenocortical tumours, testicular cancer, PPNAD and Carney Complex.

Adrenocortical tumours

Adrenocortical tumours associated with an inactivating point mutation in the gene encoding PDE11A4 have decreased expression of PDE11A4 and increased cAMP levels (Horvath, A. *et al.*, *Nat Genet.* 38: 794-800, 2006; Horvath, A. *et al.*, *Cancer Res.* 66: 11571-11575, 2006; Libé, R., *et al.*, *Clin. Cancer Res.* 14: 4016-4024, 2008).

Testicular Cancer

Mutations that reduce PDE11A activity and increase cAMP levels have been observed in some forms of testicular cancer (Horvath. A. *et al.*, *Cancer Res.* 69: 5301-5306, 2009).

Primary pigmented nodular adrenocortical diseases (PPNAD)

Mutations in the *PDE8B* gene have also been identified as a predisposing factor for PPNAD and the mutant protein shows reduced ability to degrade cAMP (Horvath, A., Mericq, V. and Stratakis, C. A. *N. Engl. J. Med.* 358: 750-752, 2008; Horvath, A. *et al.*, *Eur. J. Hum. Genet.* 16: 1245-1253, 2008).

Carney Complex

In Carney Complex (CNC) caused by PRKAR1A mutations, some patients also have defects in PDE11A that may exert a synergistic effect to enhance abnormal activation of the cAMP signal transduction pathway, leading to adrenal and testicular cancer (Libé, R. *et al.*, *J. Clin. Endocrinol. Metab.* 96: E208-214, 2011).

Treatment and posology

By "treatment" herein is meant the treatment by therapy, whether of a human or a non-human animal (e.g., in veterinary applications) typically a non-human mammal, in which some desired therapeutic effect on the condition is achieved; for example, the inhibition of the progress of the disorder, including a reduction in the rate of progress, a halt in the rate of progress, amelioration of the disorder or cure of the condition. Treatment as a prophylactic

measure is also included. References herein to prevention or prophylaxis herein do not indicate or require complete prevention of a condition; its manifestation may instead be reduced or delayed via prophylaxis or prevention according to the present invention.

By a "therapeutically effective amount" herein is meant an amount of the one or more
5   compounds of the invention or a pharmaceutical formulation comprising such one or more compounds, which is effective for producing such a therapeutic effect, commensurate with a reasonable benefit/risk ratio.

It will be appreciated that appropriate dosages of the compounds of the invention may vary from patient to patient. Determining the optimal dosage will generally involve the balancing
10   of the level of therapeutic benefit against any risk or deleterious side effects of the treatments of the present invention. The selected dosage level will depend on a variety of factors including the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds or materials used in combination and the age, sex, weight, condition,
15   general health and prior medical history of the patient. The amount of compound(s) and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve local concentrations at the site of action so as to achieve the desired effect. Administration *in vivo* can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective
20   means and dosage of administration are well known to a person skilled in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

25   In general, a suitable dose of the one or more compounds of the invention may be in the range of about 0.001 to 50 mg/kg body weight of the subject per day, preferably in a dosage

of 0.01-25 mg per kg body weight per day, e.g., 0.01, 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, 10 or

25 mg/kg per day. Where the compound(s) is a salt, solvate, prodrug or the like, the amount

administered may be calculated on the basis of the parent compound and so the actual

weight to be used may be increased proportionately.

5

Combination therapies

The compounds of the invention may also find application in mimicking or enhancing the

10   effects of drugs known to produce their therapeutic effect through lowering of intracellular

cAMP levels.

A number of therapeutically beneficial drugs have a primary mode of action involving

lowering intracellular cAMP levels and/or cAMP-mediated activity, as summarised below.

Since PDE4 long form activators of the present invention will also act to lower cAMP levels it

15   is expected that these agents will mimic and / or augment the pharmacological properties

and therapeutic utility of drugs operating through a down-regulation of cAMP-mediated

signalling. In certain embodiments, a compound of the invention is therefore provided as

part of a combination therapy with another agent that lowers intracellular cAMP levels and/or

cAMP-mediated activity. The combination therapy may be administered simultaneously,

20   contemporaneously, sequentially or separately. In one embodiment, the compound of the

invention and the separate cAMP lowering agent are provided in a single composition, as

described in more detail below. The combination therapy may comprise a compound of the

invention and one or more of:

           (i)   a presynaptic α-2 adrenergic receptor agonist, optionally clonidine,

25   dexmedetomidine, or guanfacine;

(ii)   a β-1 Adrenergic receptor antagonist ("beta-blocker"), optionally Atenolol, Metoprolol, Bisoprolol, Acebutolol, or Betaxolol.

Combination with α-2 Adrenergic receptor agonist

α-2 Adrenergic receptor stimulation is known to reduce cAMP levels through a G_i protein-
5   mediated inhibition of adenylyl cyclase activity in a broad range of tissues. In noradrenergic neurones in the brain and peripheral sympathetic nervous system, presynaptic α-2 adrenergic receptor activation inhibits noradrenaline release and noradrenergic activity. Drugs (e.g. clonidine, dexmedetomidine, guanfacine) that act as agonists at these receptors are effective in the treatment of a variety of clinical conditions. Clonidine, the prototypic
10   agent, has shown therapeutic utility in the treatment of hypertension, neuropathic pain, opioid detoxification, insomnia, ADHD, Tourette syndrome, sleep hyperhidrosis, addiction (narcotic, alcohol and nicotine withdrawal symptoms), migraine, hyperarousal, anxiety and also as a veterinary anaesthetic. Lowering of cAMP levels by PDE4 long form activation may be expected to yield similar effects to drugs acting through α-2 adrenergic receptor
15   stimulation. Furthermore, PDE4 long form activators of the present invention may be expected to potentiate the pharmacodynamic effects of α-2 adrenergic receptor agonists when used in combination.

Combination with β-1 Adrenergic receptor antagonist

β-1 Adrenergic receptor antagonists are used in the treatment a range of cardiovascular
20   indications including hypertension, cardiac arrhythmias and cardioprotection following myocardial infarction. Their primary mechanism of action involves reducing the effects of excessive circulating adrenaline and sympathetic activity, mediated by noradrenaline, particularly at cardiac β-1 adrenergic receptors. Endogenous and synthetic β-1 adrenergic receptor agonists stimulate adenylyl cyclase activity through G_s activation and raise
25   intracellular cAMP levels in a variety of tissues such as heart and kidney. Consequently, drugs that block β-1 adrenergic receptor mediated activity exert their pharmacological effects

by attenuating the increase in cAMP mediated signalling. Given that PDE4 long form activation will also lower cAMP concentration and transduction in cardiac tissue, PDE4 long form activators of the present invention may be expected to find utility in the treatment or partial control of hypertension, cardiac arrhythmias, congestive heart failure and

5   cardioprotection. Additional non-cardiovascular therapeutic utility may be expected in disorders such as post-traumatic stress related disorder, anxiety, essential tremor and glaucoma, which also respond to β-1 adrenergic antagonist treatment. Furthermore, PDE4 long form activators of the present invention may be expected to potentiate the pharmacodynamic effects of β-1 adrenergic receptor antagonists when used in combination.

10   Methods of treatment

In a further aspect, the present invention provides a small molecule activator of a PDE4 long form of Formula 1 or Formula 2 for use in a method for the treatment or prevention of a disease or disorder in a patient in need of such therapy. The disease or disorder may be any disease of disorder described herein, including: a disease associated with increased

15   cAMP production and signalling (such as hyperthyroidism, Jansens's metaphyseal chondrodysplasia, hyperparathyroidism, familial male-limited precocious puberty, pituitary adenomas, Cushing's disease, polycystic kidney disease, polycystic liver disease, MODY5 and cardiac hypertrophy); diseases known to be associated with increased cAMP-mediated signalling, including disorders associated with activating mutations of the alpha subunit of

20   the G protein (GNAS1) (such as McCune-Albright syndrome); amelioration of toxin-induced increases in adenylyl cyclase activity in infectious diseases (such as cholera, whooping cough, anthrax, and tuberculosis); treatment of diseases known to be dependent upon activation of PKA by elevated cAMP (such as HIV infection and AIDS, and Common Variable Immunodeficiency (CVID)); treatment of diseases known to be dependent upon activation of

25   either or both of Epac1 and Epac2 by elevated cAMP (such as melanoma and pancreatic cancer); treatment of diseases dependent upon modulation of cAMP-gated ion channels by elevated cAMP; treatment of diseases known to be associated with increased activity of

cAMP response element binding protein (such as leukaemia and prostate cancer); treatment of diseases known to be associated with reduced activity of cAMP-hydrolysing PDE enzymes (such as adrenocortical tumours, testicular cancer, primary pigmented nodular adrenocortical diseases (PPNAD) and Carney Complex); and mimicking or enhancing the effects of drugs known to produce their therapeutic effect through lowering of intracellular cAMP levels.

As used herein, the terms "compound of the invention", "compound of Formula 1", "compound of Formula 2" *etc.* include pharmaceutically acceptable derivatives thereof and polymorphs, isomers and isotopically labelled variants thereof. Furthermore, these terms include all the sub-embodiments of those compounds disclosed herein.

Pharmaceutically acceptable derivatives

The present invention further provides pharmaceutical compositions comprising a compound of the invention, including a pharmaceutically acceptable salt, solvate, ester, hydrate or amide thereof, in admixture with a pharmaceutically acceptable excipient(s), and optionally other therapeutic agents. The term "acceptable" means being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. Compositions include e.g. those suitable for oral, sublingual, subcutaneous, intravenous, epidural, intrathecal, intramuscular, transdermal, intranasal, pulmonary, topical, local, or rectal administration, and the like, typically in unit dosage forms for administration.

The term "pharmaceutically acceptable salt" includes a salt prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic or organic acids and bases. Compounds of the invention which contain basic, *e.g.* amino, groups are capable of forming pharmaceutically acceptable salts with acids. Examples of pharmaceutically acceptable acid addition salts of the compounds according to the invention include acid addition salts formed with organic carboxylic acids such as acetic, lactic, tartaric, maleic, citric, pyruvic, oxalic, fumaric, oxaloacetic, isethionic, lactobionic and succinic acids; organic sulfonic acids such

as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids.

Compounds of the invention which contain acidic, *e.g.* carboxyl, groups are capable of forming pharmaceutically acceptable salts with bases. Pharmaceutically acceptable basic salts of the compounds of the invention include, but are not limited to, metal salts such as alkali metal or alkaline earth metal salts (*e.g.* sodium, potassium, magnesium or calcium salts) and zinc or aluminium salts and salts formed with ammonia or pharmaceutically acceptable organic amines or heterocyclic bases such as ethanolamines (*e.g.* diethanolamine), benzylamines, N-methyl-glucamine, amino acids (*e.g.* lysine) or pyridine.

Hemisalts of acids and bases may also be formed, *e.g.* hemisulphate salts.

Pharmaceutically acceptable salts of compounds of the compounds of the invention may be prepared by methods well-known in the art. For a review of pharmaceutically acceptable salts, see Stahl and Wermuth, Handbook of Pharmaceutical Salts: Properties, Selection and Use (Wiley-VCH, Weinheim, Germany, 2002).

Prodrugs

The invention includes prodrugs of the compounds of Formulae 1 and 2. Prodrugs are derivatives of compounds of Formula 1 or 2 (which may have little or no pharmacological activity themselves), which can, when administered *in vivo*, be converted into compounds of Formula 1 or 2.

Prodrugs can, for example, be produced by replacing functionalities present in the compounds of Formula 1 or 2 with appropriate moieties which are metabolized *in vivo* to form a compound of Formula 1 or 2. The design of prodrugs is well-known in the art, as discussed in Bundgaard, *Design of Prodrugs* **1985** (Elsevier), *The Practice of Medicinal Chemistry* **2003**, 2nd Ed, 561-585 and Leinweber, *Drug Metab. Res.* **1987**, 18: 379.

Examples of prodrugs of compounds of Formula 1 or 2 are amides and esters of those compounds. For example, where the compound of Formula 1 or 2 contains a carboxylic acid group (-COOH), the hydrogen atom of the carboxylic acid group may be replaced in order to form an ester (*e.g.* the hydrogen atom may be replaced by C_{1-6}alkyl). Where a compound

5   contains an alcohol group (-OH), the hydrogen atom of the alcohol group may be replaced in order to form an ester (*e.g.* the hydrogen atom may be replaced by $-C(O)C_{1-6}$alkyl.

Solvates

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the compounds described herein, which may be used in the any one of the uses/methods

10   described. The term solvate is used herein to refer to a complex of solute, such as a compound or salt of the compound, and a solvent. If the solvent is water, the solvate may be termed a hydrate, for example a mono-hydrate, di- hydrate, tri-hydrate etc, depending on the number of water molecules present per molecule of substrate.

Stereoisomers

15   It will be appreciated that the compounds of the present invention may exist in various stereoisomeric forms and the compounds of the present invention as hereinbefore defined include all stereoisomeric forms and mixtures thereof, including enantiomers and racemic mixtures. The present invention includes within its scope the use of any such stereoisomeric form or mixture of stereoisomers, including the individual enantiomers of the compounds of

20   Formula 1 or 2 as well as wholly or partially racemic mixtures of such enantiomers. Where appropriate isomers can be separated from their mixtures by the application or adaptation of known methods (*e.g.* chromatographic techniques and recrystallisation techniques). Where appropriate isomers can be prepared by the application or adaptation of known methods (*e.g.* asymmetric synthesis).

25   Isotopes

The invention includes pharmaceutically acceptable isotopically-labelled compounds of Formula 1 or 2 wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature.

5　Examples of isotopes suitable for inclusion in the compounds of the invention include isotopes of hydrogen, such as ^{2}H and ^{3}H, carbon, such as ^{11}C, ^{13}C and ^{14}C, chlorine, such as ^{36}Cl, fluorine, such as ^{18}F, iodine, such as ^{123}I and ^{125}I, nitrogen, such as ^{13}N and ^{15}N, oxygen, such as ^{15}O, ^{17}O and ^{18}O, and sulphur, such as ^{35}S. Certain isotopically-labelled compounds of Formula 1 or 2, for example, those incorporating a radioactive isotope, are

10　useful in drug and/or substrate tissue distribution studies. The radioactive isotopes ^{3}H and ^{14}C are particularly useful for this purpose in view of their ease of incorporation and ready means of detection. Substitution with positron emitting isotopes, such as ^{11}C, ^{18}F, ^{15}O and ^{13}N, can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labelled compounds of Formula 1 or 2 can generally be

15　prepared by conventional techniques known to those skilled in the art or by processes analogous to those described herein using an appropriate isotopically-labelled reagent in place of the non-labelled reagent previously employed.

Pharmaceutical compositions

For oral administration, the active ingredient may be presented as discrete units, such as

20　tablets, capsules, powders, granulates, solutions, suspensions, and the like.

Formulations suitable for oral administration may also be designed to deliver the compounds of the invention in an immediate release manner or in a rate-sustaining manner, wherein the release profile can be delayed, pulsed, controlled, sustained, or delayed and sustained or modified in such a manner which optimises the therapeutic efficacy of the said compounds.

25　Means to deliver compounds in a rate-sustaining manner are known in the art and include

slow release polymers that can be formulated with the said compounds to control their release.

Examples of rate-sustaining polymers include degradable and non-degradable polymers that can be used to release the said compounds by diffusion or a combination of diffusion and polymer erosion. Examples of rate-sustaining polymers include hydroxypropyl methylcellulose, hydroxypropyl cellulose, methyl cellulose, ethyl cellulose, sodium carboxymethyl cellulose, polyvinyl alcohol, polyvinyl pyrrolidone, xanthum gum, polymethacrylates, polyethylene oxide and polyethylene glycol.

Liquid (including multiple phases and dispersed systems) formulations include emulsions, suspensions, solutions, syrups and elixirs. Such formulations may be presented as fillers in soft or hard capsules (made, for example, from gelatin or hydroxypropylmethylcellulose) and typically comprise a carrier, for example, water, ethanol, polyethylene glycol, propylene glycol, methylcellulose, or a suitable oil, and one or more emulsifying agents and/or suspending agents. Liquid formulations may also be prepared by the reconstitution of a solid, for example, from a sachet.

The compounds of the invention may also be used in fast-dissolving, fast-disintegrating dosage forms such as those described in Liang and Chen, *Expert Opinion in Therapeutic Patents* **2001**, 11(6): 981-986.

The formulation of tablets is discussed in H. Lieberman and L. Lachman, *Pharmaceutical Dosage Forms: Tablets* **1980**, vol. 1 (Marcel Dekker, New York).

For administration intranasally or by inhalation, the active ingredient may be presented in the form of a dry powder from a dry powder inhaler or in the form of an aerosol spray of a solution or suspension from a pressurised container, pump, spray, atomiser or nebuliser.

For parenteral administration, the pharmaceutical composition of the invention may be presented in unit-dose or multi-dose containers, e.g. injection liquids in predetermined

amounts, for example in sealed vials and ampoules, and may also be stored in a freeze dried (lyophilized) condition requiring only the addition of sterile liquid carrier, e.g. water, prior to use.

For parenteral administration, the compounds of the invention may be administered directly

5   into the blood stream, into subcutaneous tissue, into muscle, or into an internal organ. Suitable means for administration include intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular, intrasynovial and subcutaneous. Suitable devices for administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques.

10   Parenteral formulations are typically aqueous or oily solutions. Where the solution is aqueous, excipients such as sugars (including but restricted to glucose, mannitol, sorbitol, *etc.*) salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-

15   free water (WFI).

Parenteral formulations may include implants derived from degradable polymers such as polyesters (*i.e.* polylactic acid, polylactide, polylactide-co-glycolide, polycapro-lactone, polyhydroxybutyrate), polyorthoesters and polyanhydrides. These formulations may be administered *via* surgical incision into the subcutaneous tissue, muscular tissue or directly

20   into specific organs.

The preparation of parenteral formulations under sterile conditions, for example, by lyophilisation, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art.

The solubility of compounds of the invention used in the preparation of parenteral solutions

25   may be increased by the use of appropriate formulation techniques, such as the

incorporation of co-solvents and/or solubility-enhancing agents such as surfactants, micelle structures and cyclodextrins.

Mixed with such pharmaceutically acceptable excipients, e.g. as described in the standard reference, Gennaro, A.R. et al, Remington: The Science and Practice of Pharmacy (21st

5   Edition, Lippincott Williams & Wilkins, 2005, see especially Part 5: Pharmaceutical Manufacturing), the active agent may be compressed into solid dosage units, such as pills, tablets, or be processed into capsules, suppositories or patches. By means of pharmaceutically acceptable liquids the active agent can be applied as a fluid composition, e.g. as an injection preparation or as an aerosol spray, in the form of a solution, suspension,

10   or emulsion.

For making solid dosage units, the use of conventional additives such as fillers, colorants, polymeric binders and the like is contemplated. In general any pharmaceutically acceptable additive that does not interfere with the function of the active compounds can be used. Suitable carriers with which the active agent of the invention can be administered as solid

15   compositions include lactose, starch, cellulose derivatives and the like, or mixtures thereof, used in suitable amounts. For parenteral administration, aqueous suspensions, isotonic saline solutions and sterile injectable solutions may be used, containing pharmaceutically acceptable dispersing agents and/or wetting agents, such as propylene glycol or butylene glycol.

20   The invention further includes a pharmaceutical composition, as hereinbefore described, in combination with packaging material suitable for said composition, said packaging material including instructions for the use of the composition for the use as hereinbefore described.

In some embodiments, the one or more compounds of the present invention may be used in combination therapies for the treatment of the described conditions i.e., in conjunction with

25   other therapeutic agents. For the case of active compounds combined with other therapies

the two or more treatments may be given in individually varying dose schedules and *via* different routes.

The combination of the agents listed above with a compound of the present invention would be at the discretion of the physician who would select dosages using his common general

5   knowledge and dosing regimens known to a skilled practitioner.

Where a compound of the invention is administered in combination therapy with one, two, three, four or more, preferably one or two, preferably one other therapeutic agents, the compounds can be administered simultaneously or sequentially. When administered sequentially, they can be administered at closely spaced intervals (for example over a period

10 of 5-10 minutes) or at longer intervals (for example 1, 2, 3, 4 or more hours apart, or even longer period apart where required), the precise dosage regimen being commensurate with the properties of the therapeutic agent(s).

In one embodiment, the invention provides a product comprising a compound of the invention and another therapeutic agent as a combined preparation for simultaneous,

15 separate or sequential use in therapy. In one embodiment, the therapy is the treatment or prevention of disorders where a reduction of second messenger responses mediated by cyclic 3',5'-adenosine monophosphate (cAMP) is required. Products provided as a combined preparation include a composition comprising a compound of the invention and the other therapeutic agent together in the same pharmaceutical composition, or the compound of the

20 invention and the other therapeutic agent in separate form, *e.g.* in the form of a kit.

In one embodiment, the invention provides a pharmaceutical composition comprising a compound of the invention and another therapeutic agent. Optionally, the pharmaceutical composition may comprise a pharmaceutically acceptable excipient, as described above.

In one embodiment, the invention provides a kit comprising two or more separate

25 pharmaceutical compositions, at least one of which contains a compound of the invention. In one embodiment, the kit comprises means for separately retaining said compositions, such

as a container, divided bottle, or divided foil packet. An example of such a kit is a blister pack, as typically used for the packaging of tablets, capsules and the like.

The kit of the invention may be used for administering different dosage forms, for example, oral and parenteral, for administering the separate compositions at different dosage intervals, or for titrating the separate compositions against one another. To assist compliance, the kit of the invention typically comprises directions for administration.

In the combination therapies of the invention, the compound of the invention and the other therapeutic agent may be manufactured and/or formulated by the same or different manufacturers. Moreover, the compound of the invention and the other therapeutic may be brought together into a combination therapy: (i) prior to release of the combination product to physicians (*e.g.* in the case of a kit comprising the compound of the invention and the other therapeutic agent); (ii) by the physician themselves (or under the guidance of the physician) shortly before administration; (iii) in the patient themselves, *e.g.* during sequential administration of the compound of the invention and the other therapeutic agent.

15   Method of manufacture & method of treatment

The invention also provides the use of a compound of the invention in the manufacture of a medicament for the treatment or prevention of disorders where a reduction of second messenger responses mediated by cyclic 3',5'-adenosine monophosphate (cAMP) is required, wherein the medicament is prepared for administration with another therapeutic agent. The invention also provides the use of another therapeutic agent in the manufacture of medicament for treating a disease or condition mediated by cAMP for the treatment or prevention of disorders where a reduction of second messenger responses mediated by cyclic 3',5'-adenosine monophosphate (cAMP) is required, wherein the medicament is prepared for administration with a compound of the invention.

The invention also provides a compound of the invention for use in the treatment or prevention of disorders where a reduction of second messenger responses mediated by

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cyclic 3′,5′-adenosine monophosphate (cAMP) is required, wherein the compound of the invention is prepared for administration with another therapeutic agent. The invention also provides another therapeutic agent for use in the treatment or prevention of disorders where a reduction of second messenger responses mediated by cyclic 3′,5′-adenosine

5   monophosphate (cAMP) is required, wherein the other therapeutic agent is prepared for administration with a compound of the invention. The invention also provides a compound of the invention for use in for the treatment or prevention of disorders where a reduction of second messenger responses mediated by cyclic 3′,5′-adenosine monophosphate (cAMP) is required, wherein the compound of the invention is administered with another therapeutic

10   agent. The invention also provides another therapeutic agent for use in the treatment or prevention of disorders where a reduction of second messenger responses mediated by cyclic 3′,5′-adenosine monophosphate (cAMP) is required, wherein the other therapeutic agent is administered with a compound of the invention.

The invention also provides the use of a compound of the invention in the manufacture of a

15   medicament for the treatment or prevention of disorders where a reduction of second messenger responses mediated by cyclic 3′,5′-adenosine monophosphate (cAMP) is required, wherein the patient has previously (e.g. within 24 hours) been treated with another therapeutic agent. The invention also provides the use of another therapeutic agent in the manufacture of a medicament for the treatment or prevention of disorders where a reduction

20   of second messenger responses mediated by cyclic 3′,5′-adenosine monophosphate (cAMP) is required, wherein the patient has previously (e.g. within 24 hours) been treated with a compound of the invention.

In one embodiment, the other therapeutic agent is:

(i)   a presynaptic α-2 adrenergic receptor agonist, optionally clonidine,

25   dexmedetomidine, or guanfacine;

(ii)   a β-1 Adrenergic receptor antagonist ("beta-blocker"), optionally Atenolol, Metoprolol, Bisoprolol, Acebutolol, or Betaxolol.

Examples

The present invention will now be further described by way of the following non-limiting

5   examples and with reference to the Tables and Figures:

Table 1 shows examples of novel small molecule PDE4 long form activators of Formula 1 and Formula 2 (Examples A to L), according to the present invention;

Table 2 shows activation of PDE4D5, a long form of PDE4, by Examples A to L.

Table 3 shows activation of PDE4A4, another long form of PDE4, by Example A.

10   Table 4 shows activation of PDE4B1, another long form of PDE4, by Example A.

Figure 1 shows activation of PDE4D5, a long form of PDE4, by Examples A, B, D and L.

Figure 2 shows activation of PDE4A4, another long form of PDE4, by Example A.

Figure 3 shows activation of PDE4B1, another long form of PDE4, by Example A.

Figure 4 shows a lack of effect on PDE4B2, a short form of PDE4, Examples A, B, D and L.

15   Figure 5 shows a reduction in intracellular cAMP levels in HEK 293 cells treated with a PDE4 long form activator of the present invention (10 µM) for 10 min prior to forskolin (F) (10 µM) for 2 min.

Figure 6 shows a reduction in intracellular cAMP levels in Madin-Darby canine kidney (MDCK) cells treated with PDE4 long form activators of the present invention (10 µM) for 10

20   min prior to forskolin (F) (10 µM) for 2 min.

Figure 7 shows inhibition of *in vitro* cyst formation in MDCK cells treated with a PDE4 long form activator of the present invention.

Figure 8 shows reversal of *in vitro* cyst formation in MDCK cells treated with a PDE4 long form activator of the present invention.

Figure 9 shows inhibition of proliferation of androgen-sensitive (AS) LNCaP human prostate cancer cells treated with a PDE4 long form activator of the present invention.

5   Figure 10 shows inhibition of proliferation of androgen-insensitive (AI) LNCaP human prostate cancer cells treated with a PDE4 long form activator of the present invention.

Figure 11 shows the *in vivo* mouse pharmacokinetic profile of Example A, determined by whole blood analysis at defined time points after i.v. and p.o. administration to male C57 mice.

10   Figure 12 shows the *in vivo* rat pharmacokinetic profile of Example A, determined by blood plasma analysis at defined time points after i.v. and p.o. administration to male SD rats.

Figure 13 shows (A) inhibition and (B) reversal of *in vitro* cyst formation in OX161 cells treated with Example A, a PDE4 long form activator of the present invention.

15   Experimental details

Preparation of novel PDE4 long form activators of Formula 1 and Formula 2

Reactions were monitored by thin layer chromatography (Merck Millipore TLC Silica Gel 60 F_{254}). Flash column chromatography was performed on Phenomenex Strata® pre-packed silica gel columns. NMR spectra were recorded using a Bruker AV300 spectrometer at 25

20   °C. The following abbreviations are used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), dd (doublet of doublet), dt (doublet of triplet).

Example   A: *N*-(3-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide

Step 1: N-(3-Fluorobenzyl)-2-chloroacetamide

To a stirred solution of 3-fluorobenzylamine (3.32g, 26.6 mmol) and triethylamine (3.87 mL, 27.9 mmol) in dry dichloromethane (80 mL) at -5°C (salt / ice bath) under argon was added chloroacetyl chloride (2.22 mL, 27.9 mmol), dropwise over 10 minutes. The reaction mixture

5   was stirred at -5°C for a further 30 minutes, then stirred at room temperature for 2 hours. The mixture was then diluted with chloroform (50 mL), washed with saturated aqueous sodium bicarbonate solution (3 x 20 mL) and then brine (3 x 20 mL). The organic layer was then dried over anhydrous sodium sulfate, filtered through a 2cm silica gel pad, washing with 2% methanol in chloroform, and the filtrate concentrated under reduced pressure to afford N-

10   (3-fluorobenzyl)-2-chloroacetamide as a white solid (5.15 g, 25.5 mmol).

Step 2: N-(3-Fluorobenzyl)-2-iodoacetamide

To a stirred solution of N-(3-fluorobenzyl)-2-chloroacetamide (5.13 g, 25.4 mmol) in acetonitrile (50 mL) was added sodium iodide (4.00g, 26.7 mmol). The mixture was refluxed at 90°C (oil bath) for 3h and then allowed to cool to room temperature. The precipitate was

15   filtered off using a short pad of Celite®, washing with dichloromethane. The filtrate was concentrated under reduced pressure to afford crude product as a light brown solid. The crude product was purified by flash column chromatography, eluting with 5% to 35% ethyl acetate in petroleum ether, to afford N-(3-fluorobenzyl)-2-iodoacetamide as a pale yellow powder (7.11 g, 24.3 mmol).

20   Step 3: 4-Chloro-3-fluorobenzene carboximidic acid methyl ester, hydrochloride salt

Hydrochloric acid solution in methanol (3N; 3 mL), chlorotrimethylsilane (3.26 g, 30.0 mmol) and 4-chloro-3-fluorobenzonitrile (2.33 g, 15 mmol) were added sequentially to a dry reaction tube, with stirring under argon at room temperature. The mixture was heated to 50°C with stirring for 1h, during which time a thick white precipitate formed in the mixture. Cyclopentyl

25   methyl ether (5 mL) was added and the mixture was heated to 50°C for a further 1h, with periodic shaking to loosen the precipitate. The mixture was then allowed to cool to room

temperature and the solid filtered off, washing with cyclopentyl methyl ether (2 x 5 mL) to afford 4-chloro-3-fluorobenzene carboximidic acid methyl ester, hydrochloride salt (1.43 g, 6.9 mmol).

Step 4: 3-(4-Chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazole

5   A mixture of 4-chloro-3-fluorobenzene carboximidic acid methyl ester, hydrochloride salt (187 mg, 0.83 mmol), hydrazine hydrate (0.88 mL, 18.0 mmol) and aluminium chloride (120 mg, 0.90 mmol) in toluene (30 mL) was heated to reflux for 3h. The mixture was concentrated under reduced pressure, taken up in toluene and concentrated under reduced pressure twice more. The residue was suspended in a toluene / acetonitrile mixture (12:1, 26

10   mL), propionyl chloride (0.36 mL, 4.1 mmol) was added and the mixture was heated at 112°C overnight. The mixture was concentrated under reduced pressure and purified by flash column chromatography, eluting with 20% to 25% ethyl acetate in petroleum ether, to afford 3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazole (28 mg, 0.12 mmol).

Step   5:   *N*-(3-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

15   yl]acetamide

To a stirred solution of 3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazole (28 mg, 0.12 mmol) in dimethyl formamide (2 mL) under argon at 0°C was added sodium hydride (60% dispersion in mineral oil, 6.3 mg, 0.16 mmol). After 10 minutes, a solution of *N*-(3-fluorobenzyl)-2-iodoacetamide (46 mg, 0.16 mmol) in dimethylformamide (3 mL) was added

20   dropwise. The reaction was allowed to warm to room temperature and stirred for 72h. The mixture was concentrated under reduced pressure and then partitioned between chloroform (25 mL) and water (10 mL). The organic layer was separated, washed with brine (2 x 10 mL), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography, eluting with 25%

25   to 35% ethyl acetate in petroleum ether and the product recrystallized from chloroform /

methanol to afford *N*-(3-fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide as a white solid (15.3 mg, 0.039 mmol).

¹H NMR: δ$_H$ (300 MHz, CDCl₃) 7.89 – 7.80 (2H, m), 7.56 – 7.43 (1H, m), 7.38 – 7.22 (1H, m), 7.00 (3H, m), 6.62 (1H, s), 4.87 (2H, s), 4.50 (2H, d, *J* 5.9), 2.85 (2H, q, *J* 7.6), 1.41 (3H, t, *J* 7.6).

Example B: *N*-Benzyl-2-[3-(4-chlorophenyl)-5-methoxymethyl-1*H*-1,2,4-triazol-1-yl]acetamide

Step 1: *N*-Benzyl-2-chloroacetamide

To a stirred solution of benzylamine (7.64 mL, 70.0 mmol) and triethylamine (10.22 mL, 73.5 mmol) in toluene (70 mL) at -5°C (salt / ice bath) was added chloroacetyl chloride (5.85 mL, 73.5 mmol), dropwise over 10 minutes. The reaction mixture was stirred at -5°C for a further 30 minutes, then stirred at room temperature for 2 hours. The mixture was then diluted with ethyl acetate (70 mL), washed with saturated aqueous sodium bicarbonate solution (3 x 30 mL) and brine (3 x 30 mL). The organic layer was then dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford *N*-benzyl-2-chloroacetamide as a white solid (11.01 g, 59.96 mmol).

Step 2: *N*-Benzyl-2-iodoacetamide

To a stirred solution of *N*-benzyl-2-chloroacetamide (12.43 g, 67.66 mmol) in acetonitrile (60 mL) was added sodium iodide (10.65 g, 71.04 mmol). The mixture was gently refluxed at 95ºC (oil bath) for 2.5 hours and then allowed to cool to room temperature. The precipitate was filtered off using a short pad of Celite®, washing with ethyl acetate. The filtrate was concentrated under reduced pressure to afford crude product as a light brown solid. Trituration with an ethyl acetate / dichloromethane mixture followed by filtration afforded N-benzyl-2-iodoacetamide (3.91 g). The filtrate was concentrated under reduced pressure and purified by flash column chromatography eluting with 50% chloroform / ethyl acetate to afford further product (2.35 g). The pad of Celite® was further washed with dichloromethane and

then chloroform to afford further product (7.10 g). The product samples were combined and dried in air to afford a single batch of *N*-benzyl-2-iodoacetamide as a white powder (13.14 g, 47.76 mmol).

Step 3: 3-(4-Chlorophenyl)-5-methoxymethyl-1*H*-1,2,4-triazole

5   To a solution of sodium methoxide in dry methanol (0.5M; 4 mL) was added 4-chlorobenzonitrile (1.38 g, 10 mmol). The resulting suspension was stirred at room temperature under argon for 2.5h. A solution of methoxyacetic acid hydrazide (1.04 g, 10 mmol) in dry methanol (10 mL) was added to the mixture, resulting in a clear solution, which was heated to reflux for 3h and then stirred at room temperature overnight. The mixture was

10   concentrated under reduced pressure and purified by flash column chromatography eluting with 20% to 60% ethyl acetate in petroleum ether to afford 3-(4-chlorophenyl)-5-methoxymethyl-1*H*-1,2,4-triazole as a white solid (412 mg, 1.84 mmol).

Step 4: *N*-Benzyl-2-[3-(4-chlorophenyl)-5-methoxymethyl-1*H*-1,2,4-triazol-1-yl]acetamide

To a stirred solution of 3-(4-chlorophenyl)-5-methoxymethyl-1*H*-1,2,4-triazole (45 mg, 0. 20

15   mmol) in dimethyl formamide (2 mL) under argon at 0°C was added sodium hydride (60% dispersion in mineral oil, 9.6 mg, 0.24 mmol). After 10 minutes, a solution of *N*-benzyl-2-iodoacetamide (66 mg, 0.24 mmol) in dimethylformamide (3 mL) was added dropwise. The reaction was allowed to warm to room temperature and stirred for 72h. The mixture was concentrated under reduced pressure and then partitioned between dichloromethane (25

20   mL) and water (10 mL). The organic layer was separated, washed with brine (2 x 10 mL), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography, eluting with 40% to 50% ethyl acetate in petroleum ether and the product recrystallized from chloroform / methanol / hexane to afford *N*-benzyl-2-[3-(4-chlorophenyl)-5-methoxymethyl-1*H*-1,2,4-

25   triazol-1-yl]acetamide as a white solid (22 mg, 0.059 mmol).

¹H NMR: δ$_H$ (300 MHz, CDCl₃) 8.07 – 7.96 (2H, m), 7.49 – 7.39 (2H, m), 7.38 – 7.19 (5H, m), 6.48 (1H, s), 5.01 (2H, s), 4.70 (2H, s), 4.50 (2H, d, J 5.8), 3.40 (3H, s).

Example C: N-Benzyl-2-[3-(4-chlorophenyl)-5-methyl-1H-1,2,4-triazol-1-yl]acetamide

The title compound was prepared according to the method of Example B, using
5   acethydrazide instead of methoxyacetic acid hydrazide.

¹H NMR: δ$_H$ (300 MHz, CDCl₃) 8.05 – 7.93 (2H, m), 7.47 – 7.39 (2H, m), 7.38 – 7.23 (5H, m), 6.64 (1H, s), 4.85 (2H, s), 4.50 (2H, d, J 5.8), 2.55 (3H, s).

Example D: N-(3-Fluorobenzyl)-2-{3-[4-(trifluoromethoxy)phenyl]-5-methoxymethyl-1H-1,2,4-triazol-1-yl}acetamide

10   The title compound was prepared according to the method of Example B, steps 3 and 4, using 4-(trifluoromethoxy)benzonitrile instead of 4-chlorobenzonitrile and N-(3-fluorobenzyl)-2-iodoacetamide (as prepared in Example A) instead of N-benzyl-2-iodoacetamide.

¹H NMR: δ$_H$ (300 MHz, CDCl₃) 8.08–8.12 (2H, m), 7.19–7.34 (3H, m), 6.91–7.01 (3H, m), 6.45 (1H, bs), 5.00 (2H, s), 4.68 (2H, s), 4.46 (2H, d, J 5.9), 3.42 (3H, s).

15   Example E: N-(3-Chlorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide

Step 1: 3-(4-Chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazole

The triazole was prepared according to the method of Example B, step 3, using 4-chloro-3-fluorobenzonitrile instead of 4-chlorobenzonitrile and propionic acid hydrazide instead of
20   methoxyacetic acid hydrazide.

Step 2: O-tert-butyl-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetate

To a stirred solution of 3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazole (492 mg, 2.18 mmol) in dimethylformamide (10 mL) at room temperature under argon was added sodium

hydride (60% dispersion in mineral oil, 105 mg, 2.62 mmol). After 10 minutes, *tert*-butylbromoacetate (386 μL, 2.62 mmol) was added dropwise over 1 minute. The reaction was stirred at room temperature for 18h. The mixture was concentrated under reduced pressure and then purified by flash column chromatography, eluting with 5% to 15% ethyl acetate in petroleum ether to afford *O-tert*-butyl-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetate as a white solid (661 mg, 1.94 mmol).

Step 3: 2-[3-(4-Chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetic acid

To a stirred solution of *O-tert*-butyl-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetate (661 mg, 1.94 mmol) in dichloromethane (30 mL) at 0°C was added trifluoroacetic acid (10 mL) dropwise over 5 minutes. The reaction was allowed to warm to room temperature and stirred for 18h. The mixture was concentrated under reduced pressure to afford crude 2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetic acid as a viscous light brown oil, which was used without further purification.

Step 4: 2-[3-(4-Chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetyl chloride

To a stirred solution of 2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetic acid (crude material from step 3, *ca.* 1.94 mmol) in dichloromethane (30 mL) was added thionyl chloride (2.32 mL, 32.0 mmol). The mixture was heated under reflux for 6h. The mixture was concentrated under reduced pressure to afford crude 2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetyl chloride (*ca.* 1.94 mmol), which was taken up in dichloromethane (33 mL) and used without further purification.

Step   5:   *N*-(3-Chlorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide

To a stirred solution of 3-chlorobenyzlamine (39 mg, 0.27 mmol) and triethylamine (63 μL, 0.45 mmol) in dichloromethane at 0°C was added 2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetyl chloride (crude material from step 4, *ca.* 0.18 mmol) in

dichloromethane (3 mL). The reaction was allowed to warm to room temperature and stirred for 18h. The mixture was concentrated under reduced pressure and then purified by flash column chromatography, eluting with 0.5% to 1% methanol in dichloromethane to afford a white solid, which was further purified by trituration with chloroform and hexane to afford *N*-(3-chlorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide as a white solid (30.4 mg, 0.075 mmol).

^{1}H NMR: δ_H (300 MHz, CDCl$_3$) 7.84 (1H, dd, *J* 9.9, 1.8), 7.79 (1H, ddd, *J* 8.3, 1.9, 0.8), 7.44 (1H, dd, *J* 8.2, 7.6), 7.19–7.25 (3H, m), 7.08–7.12 (1H, m), 6.59 (1H, bs), 4.84 (2H, s), 4.45 (2H, d, *J* 5.9), 2.82 (2H, q, *J* 7.6), 1.38 (3H, t, *J* 7.6).

Example F: *N*-(3-Cyanobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide

The title compound was prepared according to the method of Example E, using 3-cyanobenzylamine instead of 3-chlorobenzylamine in Step 5.

^{1}H NMR: δ_H (300 MHz, CDCl$_3$) 7.78–7.86 (2H, m), 7.53–7.59 (2H, m), 7.40–7.49 (3H, m), 6.71 (1H, bs), 4.85 (2H, s), 4.50 (2H, d, *J* 6.2), 2.82 (2H, q, *J* 7.6), 1.38 (3H, t, *J* 7.6).

Example G: *N*-[3-(Trifluoromethyl)benzyl]-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide

The title compound was prepared according to the method of Example E, using 3-(trifluoromethyl)benzylamine instead of 3-chlorobenzylamine in Step 5.

^{1}H NMR: δ_H (300 MHz, CDCl$_3$) 7.83 (1H, dd, *J* 9.9, 1.8), 7.78 (1H, ddd, *J* 8.3, 1.9, 0.8), 7.51–7.56 (1H, m), 7.41–7.47 (4H, m), 6.64 (1H, bs), 4.85 (2H, s), 4.53 (2H, d, *J* 6.0), 2.82 (2H, q, *J* 7.6), 1.37 (3H, t, *J* 7.6).

Example H: *N*-(3-Methoxybenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide

The title compound was prepared according to the method of Example E, using 3-methoxybenzylamine instead of 3-chlorobenzylamine in Step 5.

^{1}H NMR: δ_H (300 MHz, CDCl$_3$) 7.83 (1H, dd, J 9.9, 1.9), 7.78 (1H, ddd, J 8.3, 1.9, 0.8), 7.43 (1H, dd, J 8.2, 7.5), 7.21 (1H, d, J 8.0), 6.74–6.82 (3H, m), 6.53 (1H, bs), 4.83 (2H, s), 4.44 (2H, d, J 5.7), 3.76 (3H, s), 2.81 (2H, q, J 7.6), 1.37 (3H, t, J 7.6).

Example I: N-[3-(Trifluoromethoxy)benzyl]-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide

The title compound was prepared according to the method of Example E, using 3-(trifluoromethoxy)benzylamine instead of 3-chlorobenzylamine in Step 5.

^{1}H NMR: δ_H (300 MHz, CDCl$_3$) 7.84 (1H, dd, J 9.9, 1.8), 7.79 (1H, ddd, J 8.3, 1.9, 0.8), 7.44 (1H, dd, J 8.2, 7.5), 7.34 (1H, t, J 7.9), 7.11–7.17 (2H, m), 7.06–7.08 (1H, m), 6.61 (1H, bs), 4.85 (2H, s), 4.49 (2H, d, J 6.0), 2.81 (2H, q, J 7.6), 1.37 (3H, t, J 7.6).

Example J: N-(2-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide

The title compound was prepared according to the method of Example E, using 2-fluorobenzylamine instead of 3-chlorobenzylamine in Step 5.

^{1}H NMR: δ_H (300 MHz, CDCl$_3$) 7.84 (1H, dd, J 10.0, 1.9), 7.81–7.78 (1H, m), 7.42–7.47 (1H, m), 7.19–7.33 (2H, m), 7.00–7.12 (2H, m), 6.68 (1H, bs), 4.80 (2H, s), 4.51 (2H, d, J 5.9), 2.79 (2H, q, J 7.6), 1.35 (3H, t, J 7.6).

Example K: N-(4-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1H-1,2,4-triazol-1-yl]acetamide

The title compound was prepared according to the method of Example E, using 4-fluorobenzylamine instead of 3-chlorobenzylamine in Step 5.

^{1}H NMR: δ_H (300 MHz, CDCl$_3$) 7.83 (1H, dd, J 10.0, 1.8), 7.77 (1H, ddd, J 8.3, 1.9, 0.8), 7.44 (1H, dd, J 8.2, 7.5), 7.16–7.22 (2H, m), 6.96–7.04 (2H, m), 6.51 (1H, bs), 4.82 (2H, s), 4.43 (2H, d, J 5.9), 2.80 (2H, q, J 7.6), 1.36 (3H, t, J 7.6).

5 Example L: _N_-(3,4-Dimethoxybenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1_H_-1,2,4-triazol-1-yl]acetamide

The title compound was prepared according to the method of Example E, using 3,4-dimethoxybenzylamine instead of 3-chlorobenzylamine in Step 5.

^{1}H NMR: δ_H (300 MHz, CDCl$_3$) 7.85 (1H, dd, J 10.0, 1.8), 7.77 (1H, ddd, J 8.3, 1.9, 0.8), 7.44 (1H, dd, J 8.0, 7.7), 6.73–6.81 (3H, m), 6.45 (1H, bs), 4.82 (2H, s), 4.40 (2H, d, J 5.7), 3.86 10 (3H, s), 3.81 (3H, s), 2.81 (2H, q, J 7.6), 1.37 (3H, t, J 7.6).

Biological Assays

Cell culture

HEK 293 and MDCK cells (Public Health England, Cell Culture Collections) were maintained in DMEM supplemented with 10% (v/v) fetal bovine serum (Seralab), 2 mM L-glutamine, 15 1,000 U penicillin and 1,000 µg streptomycin (Life Technologies); termed complete media. Clonal HEK 293 cell lines were maintained in complete media supplemented with 0.6 mg/mL G418 (Enzo Life Sciences).

Experiment 1: Identification of PDE4 long form activators of the present invention using full-length human PDE4 isoforms PDE4D5, PDE4A4, PDE4B1 and PDE4B2

20 (Marchmont, R. J. and Houslay, M. D. _Biochem. J._ 187: 381-92, 1980)

Cell line generation

HEK 293 cells were transfected with pDEST^{TM} PDE4 expression vectors using Lipofectamine LTX/Plus reagent (Invitrogen) as outlined by the manufacturer and clonal isolates expanded to obtain cell lines that stably expressed the full-length human PDE4D5,

PDE4A4 and PDE4B1 long isoforms and the full length human PDE4B2 short isoform. These were called the HEK-PDE4D5, HEK-PDE4A4, HEK-PDE4B1 and HEK-PDE4B2 cell lines, respectively.

Lysate preparation (using PDE4D5 as a typical example)

5   HEK-PDE4D5 cells were seeded out in 100mm plates and incubated at 37°C in an atmosphere of 5% CO_2, 95% air. Cell lysates were prepared using KHEM buffer [50mM KCl, 10mM EGTA, 50mM HEPES (pH 7.2), 1.92mM $MgCl_2$].

To prepare the cell lysates, the 100mm plates containing the cells were placed on ice and washed with ice-cold PBS (phosphate buffered saline, pH 7.4). KHEM buffer (500μl) was

10   added to the cells. Cells were then scraped off the plate and triturated using a needle (BD MicrolanceTM 0.8, 40mm). The lysed cells were then centrifuged at 2000rpm for 10 minutes to remove cell debris and the supernatant (cell lysate containing recombinant PDE4D5) was transferred to a fresh tube and kept on ice.

Cytosol fraction preparation (using PDE4D5 as a typical example)

15   The cell lysate containing recombinant PDE4D5 was transferred into a centrifuge tube and placed into an ultracentrifuge (BECKMAN COULTER) and spun at high speed (100,000g) for 30 minutes at 4°C. The cytosol fraction was then collected and its protein amount determined using a BCA protein assay.

PDE Assay (using PDE4D5 as a typical example)

20   PDE assays were performed using a final concentration of 10 mM Tris/5 mM $MgCl_2$ and 1 μM [3H]-cAMP (Perkin Elmer) plus PDE4D5 cell lysate cytosol fraction, containing over-expressed PDE4D5, with and without test compound. Incubations were performed at 30°C for 5 minutes. The samples were then placed in a boiling water bath for 2 minutes to denature the PDE enzyme, and returned to ice. Samples were allowed to cool for 10 min

25   after which snake venom 5'-nucleotidase (25μl, 1mg/ml; Sigma) was added. The tubes were

vortexed and incubated in a water bath at 30°C for 10 minutes, to attain conversion of [3H]-5'-AMP to [3H]-adenosine, and then placed on ice. Dowex ion exchange resin (Sigma) prepared as a 1:1 Dowex: water stock was thoroughly re-suspended and diluted 2:1 with ethanol. The resulting Dowex suspension (0.4 ml) was added to each tube. Tubes were

5   vortexed to mix and then incubated on ice for at least 15 minutes before a final vortex. The Dowex resin was pelleted by centrifugation at 13,000g at 4°C for 3 minutes and 150 μl of the supernatant was removed to an Eppendorf tube containing 1 ml of ScintiSafe3 scintillation fluid (Fisher). Tubes were vortexed thoroughly to mix and the recovered [3H]-adenosine was quantified by measuring counts over 1 minute in a scintillation counter.

10   Compounds of the present invention activated the PDE4 long forms PDE4D5, PDE4A4 and PDE4B1 in a concentration dependent manner. Under the same assay conditions, compounds of the present invention did not activate the PDE4 short form, PDE4B2.

Data are shown in graphical form in Figures 1 to 4.

Experiment 2: Reduction of intracellular cAMP levels in HEK 293 or MDCK cells by PDE4

15   long form activators

HEK 293 or MDCK cells were seeded at 100,000 cells per well, and left to adhere overnight. The cells were then treated with the compound indicated (10 μM) for 10 minutes, prior to stimulation with forskolin (10 μM, Sigma) for 2 minutes. Media was aspirated, and hydrochloric acid (0.1M) was added to lyse the cells. The cAMP assay (Enzo Life Sciences)

20   was performed according to the manufacturer's instructions.

Compounds of the present invention reduced intracellular cAMP levels in forskolin stimulated HEK 293 or MDCK cells.

Data are shown in graphical form in Figures 5 and 6.

Experiment 3: Inhibition of *in vitro* cyst formation in MDCK cells treated with PDE4 long form

25   activators

In this study, the well-established three-dimensional (3D) MDCK cell model was used to investigate the effects of PDE4 long form activators on the formation of kidney cysts and evaluate their potential in the treatment of polycystic kidney diseases. 3D cysts were generated based on the method of Mao *et al.* (Mao, Z., Streets, A. J., Ong, A. C. M. *Am. J.*

5   *Physiol. Renal Physiol.* 300(6): F1375-F1384, 2011), with some modifications. MDCK cells (50,000 cells/well) were seeded into collagen (Life Technologies; final concentration 1 mg/mL), containing 17 mM NaOH in DMEM, supplemented with 2% (v/v) FBS, 2 mM L-glutamine and 2 mM L-glutamine, 1,000 U penicillin and 1,000 µg streptomycin (DMEM-2%FBS), on ice. Upon gelling at 37ºC, 1 mL of DMEM-2%FBS was added along with the

10 test compound indicated in the presence of 10 µM forskolin (Sigma) and 1 µg/mL [Arg^8]-vasopressin acetate salt (Sigma). Media was replenished every 2 days for 20 days; at every feed, test compound, forskolin and vasopressin were added.

Phase-contrast images were obtained on the Motic microscope (x200 magnification) every 2 days for 20 days. Per condition, 10 images were taken (in duplicate) and the average cyst

15 radius measured.

Compounds of the present invention inhibited *in vitro* cyst formation in the MDCK cells in a concentration dependent manner.

The day 20 phase-contrast images and cyst radius graphs for Example A are shown in Figure 7.

20 Experiment 4: Reversal of *in vitro* cyst formation in MDCK cells treated with PDE4 long form activators

In this study, the potential of PDE4 long form activators to reverse the formation of pre-formed cysts was evaluated. The experiment was carried out according to the method of Experiment 3. For the first 10 days, forskolin and vasopressin were added at every feed

25 (every 2 days) but no test compound was added. For the next 10 days, the test compound, forskolin and vasopressin were added at every feed (every 2 days).

Phase-contrast images were obtained on the Motic microscope (x200 magnification) every 2 days for 20 days. Per condition, 10 images were taken (in duplicate) and the average cyst radius measured.

Compounds of the present invention reversed *in vitro* cyst formation in the MDCK cells in a concentration dependent manner.

The day 20 phase-contrast images and cyst radius graphs for Example A are shown in Figure 8.

Experiment 5: Inhibition of proliferation of LNCaP human prostate cancer cells

In this study, the potential utility of PDE4 long form activators in the treatment of prostate cancer was studied using the LNCaP human prostate cancer cell line. The experiments were carried out according to the method described by Henderson *et al.* (Henderson, D. J. P., Byrne, A., Dulla, K., Jenster, G., Hoffmann, R., Baillie, G. S., Houslay, M. D. *Br. J. Cancer* 110: 1278-1287, 2014).

LNCaP cell culture

Androgen-sensitive (AS) LNCaP cells were maintained in RPMI1640 supplemented with 10% FBS (Seralabs), 2mM L-glutamine and 1,000U penicillin-streptomycin. LNCaP androgen-insensitive (AI) cells were generated in-house by culturing the LNCaP-AS cells in RPMI1640 supplemented with 10% charcoal stripped FBS, 2mM L-glutamine and 1,000U penicillin-streptomycin for a minimum of four weeks. All tissue culture reagents were from Life Technologies.

Xcelligence (Roche) proliferation assay

Cell proliferation is measured as a function of changing electrical impedance. Values are represented by cell index number, a dimensionless unit of measurement representing the cell status, which increases as cells adhere to 96-well electrode plates and divide.

LNCaP AI/AS cells were plated at a density of 25,000 cells per well in a 96-well electrode plate (in triplicate), in the presence/absence of various concentrations of test compound.

Cell indices were measured every 10 minutes for up to 100 hours, analysed using RTCA software and normalised to the cell index of vehicle-treated cells (n=3).

5   The effects of Example A on proliferation of androgen-sensitive (AS) LNCaP human prostate cancer cells are shown in Figure 9.

The effects of Example A on proliferation of androgen-insensitive (AI) LNCaP human prostate cancer cells are shown in Figure 10.

Experiment 6: Measurement of _in vitro_ clearance of Example A using human hepatocytes

10   Human hepatocyte stability is considered the gold standard method for evaluating the hepatic metabolism of drugs _in vitro_. The _in vitro_ clearance of Example A was evaluated using cryopreserved human hepatocytes according to the method of Lau _et al_. (Lau, Y. Y. _et al. Drug Metab. Dispos._ 30: 1446-1454, 2002) with minor modifications.

Cryopreserved hepatocytes were thawed in a water bath at 37°C and transferred to a tube
15   containing 50 ml of hepatocyte thaw medium. The hepatocytes were centrifuged at 500 rpm for 3 min. The supernatant was removed and the hepatocytes were resuspended in medium, mixed gently and centrifuged again at 500 rpm for 3 min. The supernatant was discarded and the hepatocyte pellet was gently resuspended in medium to a final density of 2 million cells/ml.

20   Incubations were carried out at a final test compound concentration of 1 μM. Stock solutions of the compounds were prepared in DMSO and diluted to the desired concentrations before adding to the hepatocytes. Incubations were carried out with a hepatocyte concentration of 1 million cells/ml. Samples (100 μL) were incubated at 37°C for 0, 15, 30, 60 and 120 min in duplicate. At the end of the incubation time, 200 μl of acetonitrile with internal standard was
25   added and the wells were sealed.

The samples were sonicated for 2 min and centrifuged at 6000 rpm for 10 min, and 50 µL of the supernatant was transferred into a 96-well plate containing 50 µL of ultrapure water for LC-MS/MS analysis.

The samples were analysed by LC-MS/MS using a Sciex API 4000™ system. The half-life of Example A in this assay was 54 minutes, indicating that Example A is moderately stable in human hepatocytes.

Experiment 7: Pharmacokinetic profile of Example A in male C57 mice

Example A was dissolved in 5%DMA+10%Solutol+85%(10%HPBCD in water) to afford a clear dosing solution of 1 mg/mL. The dosing solution was administered to three mice at 2 mg/kg i.v. *via* the tail vein and to three further mice at 10 mg/kg p.o. *via* oral gavage. Blood samples (ca. 20 µL) were collected at 2, 5, 20 min, 1, 2, 4, 8 and 12 h after i.v. dosing and at 15, 30 min, 1, 2, 4, 8, 12 and 24 h after oral dosing. The blood samples were diluted with 3 volumes of distilled water and stored at -80°C until analysis. The samples were analysed by UPLC-MS/MS (API 5500).

Example A exhibited 66% oral bioavailability in male C57 mice, with a terminal half life of 4.9 hours after oral dosing and 4.2 hours after i.v. dosing. No abnormal effects were observed in the mice. The mean whole blood concentration-time profiles of Example A after i.v. and p.o. dosing are shown in Figure 11.

Experiment 8: Pharmacokinetic profile of Example A in male SD rats

Example A was dissolved in 5%DMA+10%Solutol+85%(10%HPBCD in saline) to afford a clear dosing solution of 1 mg/mL. The dosing solution was administered to three rats at 2 mg/kg i.v. *via* the foot dorsal vein and to three further rats at 10 mg/kg p.o. *via* oral gavage. Blood samples (ca. 150 µL) were collected at 2, 5, 20 min, 1, 2, 4, 8, 12 and 24 h after i.v. dosing and at 5, 15, 30 min, 1, 2, 4, 8, 12 and 24 h after oral dosing. The blood samples were centrifuged at 4°C (2000 g, 5 min) within 15 min of sample collection to obtain plasma

samples. Plasma samples were stored at -80°C until analysis. The samples were analysed by UPLC-MS/MS (API 4000).

Example A exhibited 100% oral bioavailability in male SD rats, with a terminal half life of 9.1 hours after oral dosing and 2.2 hours after i.v. dosing. No abnormal effects were observed in

5   the rats. The mean plasma concentration-time profiles of Example A after i.v. and p.o. dosing are shown in Figure 12.

Experiment 9: Inhibition of *in vitro* cyst formation in OX161 cells treated with PDE4 long form activators of the present invention

In this study, a human ADPKD patient-derived (OX161) cell line was used to investigate the

10   effects of PDE4 long form activators on the formation of kidney cysts *in vitro* and evaluate their potential in the treatment of polycystic kidney diseases. Conditionally immortalised OX161 cystic tubular epithelial cells were generated from human kidneys removed for clinical indications from ADPKD patients with characterised PKD1 (PC1) mutations (Parker, E., Newby, L. J., Sharpe, C. C., Rossetti, S., Streets, A. J., Harris, P. C., O'Hare, M. J., Ong,

15   A. C. M. *Kidney Int.* 72(2): 157-165, 2007).

OX161 cells (50,000 cells/well) were seeded into collagen (Life Technologies; final concentration 1 mg/mL), containing 17 mM NaOH in DMEM, supplemented with 2% (v/v) FBS, 2 mM L-glutamine and 2 mM L-glutamine, 1,000 U penicillin and 1,000 µg streptomycin (DMEM-2%FBS), on ice. Upon gelling at 37°C, 1 mL of DMEM-2%FBS was added along

20   with the test compound indicated in the presence of 10 µM forskolin. Media was replenished every 2 days for 10 days; at every feed, test compound and forskolin were added.

Phase-contrast images were obtained every 2 days for 10 days. Per condition, 10 images were taken (in duplicate) and the average cyst area calculated.

Example A inhibited *in vitro* cyst formation in the OX161 cells in a concentration dependent

25   manner.

The day 10 phase-contrast images and cyst area graphs for Example A are shown in Figure 13 (Panel A).

<u>Experiment 10: Reversal of *in vitro* cyst formation in OX161 cells treated with PDE4 long form activators of the present invention</u>
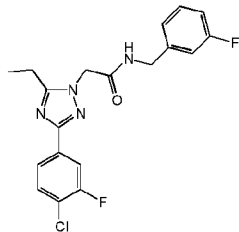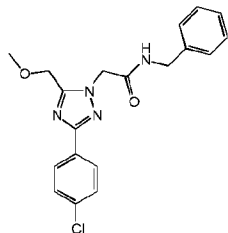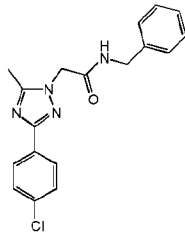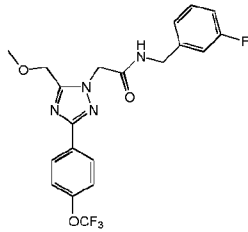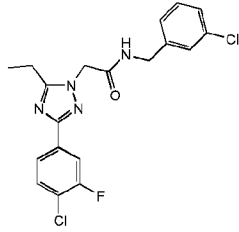
5　In this study, the potential of PDE4 long form activators to reverse the formation of preformed cysts was evaluated in a human ADPKD patient-derived OX161 cell line. The experiment was carried out according to the method of Experiment 9. For the first 10 days, forskolin was added at every feed (every 2 days) but no test compound was added. For the next 10 days, the test compound and forskolin were added at every feed (every 2 days).

10　Phase-contrast images were obtained every 2 days for 20 days. Per condition, 10 images were taken (in duplicate) and the average cyst area calculated.

Example A reversed *in vitro* cyst formation in the OX161 cells in a concentration dependent manner.
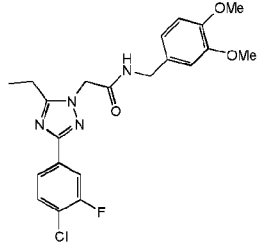
The day 20 phase-contrast images and cyst area graphs for Example A are shown in Figure 15　13 (Panel B).

Table 1: Novel small molecule PDE4 long form activators of Formula 1 and Formula 2 (Examples A to L), according to the present invention

Compound	Chemical name	Molecular weight (Daltons)	Chemical Structure
Example A	*N*-(3-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	390.8	
Example B	*N*-Benzyl-2-[3-(4-chlorophenyl)-5-methoxymethyl-1*H*-1,2,4-triazol-1-yl]acetamide	370.8	
Example C	*N*-Benzyl-2-[3-(4-chlorophenyl)-5-methyl-1*H*-1,2,4-triazol-1-yl]acetamide	340.8	
Example D	*N*-(3-Fluorobenzyl)-2-{3-[4-(trifluoromethoxy)-phenyl]-5-methoxymethyl-1*H*-1,2,4-triazol-1-yl}acetamide	438.4	
Example E	*N*-(3-Chlorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	407.3	

Compound	Chemical name	Molecular weight (Daltons)	Chemical Structure
Example F	*N*-(3-Cyanobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	397.8	
Example G	*N*-[3-(Trifluoromethyl)benzyl]-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	440.8	
Example H	*N*-(3-Methoxybenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	402.8	
Example I	*N*-[3-(Trifluoromethoxy)benzyl]-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	456.8	
Example J	*N*-(2-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	390.8	
Example K	*N*-(4-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	390.8	

Compound	Chemical name	Molecular weight (Daltons)	Chemical Structure
Example L	*N*-(3,4-Dimethoxybenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-yl]acetamide	432.9	

Using the method described in Experiment 1, the compounds shown in Table 1 were identified as PDE4 long form activators.

Table 2: Activation of PDE4D5, a long form of PDE4, by Examples A to L

5   Using the method described in Experiment 1, the following concentration / PDE4D5 activity data were obtained for Examples A to L. Data are shown in graphical form in Figure 1.

Compound	Concentration (µM)	PDE4D5 activity*	SEM
Example A	1	-3.6	6.7
Example A	3	-0.2	9.5
Example A	10	12.5	13.4
Example A	30	39.7	15.7
Example A	50	91.9	13.1
Example B	1	7.2	11.1
Example B	3	0.4	6.3
Example B	10	21.9	6.6
Example B	30	64.6	24.9
Example B	50	113.8	13.7

Compound	Concentration (µM)	PDE4D5 activity*	SEM
Example C	50	118.7	24.1
Example D	1	52.6	27.2
Example D	3	30.6	9.0
Example D	10	51.6	13.2
Example D	30	126.2	32.2
Example D	50	196.3	58.1
Example E	50	71.1	12.9
Example F	50	107.3	41.7
Example G	50	171.1	6.4
Example H	50	136.4	40.3
Example I	50	92.1	7.8
Example J	50	82.2	17.9
Example K	50	80.5	8.5
Example L	1	26.0	13.8
Example L	3	42.4	26.9
Example L	10	79.8	29.9
Example L	30	213.6	44.8
Example L	50	250.9	56.2

*Measured as mean % increase in counts over basal activity without added compound (n ≥ 2)

Table 3: Activation of PDE4A4, another long form of PDE4, by Example A

Using the method described in Experiment 1, the following concentration / PDE4A4 activity data were obtained for Example A. Data are shown in graphical form in Figure 2.

Compound	Concentration (μM)	PDE4A4 activity*	SEM
Example A	1	1.1	8.8
Example A	3	2.7	1.3
Example A	10	10.4	5.2
Example A	30	38.5	8.7
Example A	50	65.4	25.0

*Measured as mean % increase in counts over basal activity without added compound (n ≥ 2)

Table 4: Activation of PDE4B1, another long form of PDE4, by Example A

Using the method described in Experiment 1, the following concentration / PDE4B1 activity data were obtained for Example A. Data are shown in graphical form in Figure 3.

Compound	Concentration (μM)	PDE4B1 activity*	SEM
Example A	1	-2.4	3.4
Example A	3	4.9	6.3
Example A	10	22.0	5.1
Example A	30	61.3	5.8
Example A	50	92.9	7.5

*Measured as mean % increase in counts over basal activity without added compound (n ≥ 2)

CLAIMS

1. A compound of Formula 1:
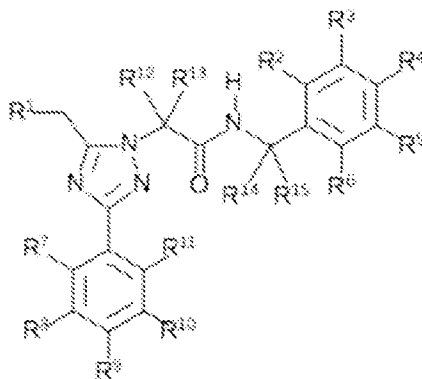
Formula 1

5   wherein

R^1 is H, (C1-4)alkyl or (C1-4)alkyloxy, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^2 and R^6 are independently selected from H, (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to

10   3 fluoros;

R^3, R^4 and R^5 are independently selected from H, (C1-4)alkyl, (C1-4)alkyloxy, (C1-4)alkylsulfonyl, C(O)-NR^{16}R^{17}, C(O)-OR^{16}, S(O)$_2$-NR^{16}R^{17}, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^7, R^8, R^{10} and R^{11} are independently selected from H and F;

15   R^9 is selected from H, (C1-4)alkyl, (C1-4)alkyloxy, (C1-4)alkylsulfonyl, C(O)-NR^{16}R^{17}, C(O)-OR^{16}, S(O)$_2$-NR^{16}R^{17}, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^{12}, R^{13}, R^{14} and R^{15} are independently selected from H and (C1-4)alkyl;

each R^{16} and R^{17}, when present, is independently selected from H and (C1-4)alkyl;

or a pharmaceutically acceptable salt thereof.

2. A compound or pharmaceutically acceptable salt of claim 1, wherein R^7 and R^{11} are H and/or wherein R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are H.

3. A compound or pharmaceutically acceptable salt of claim 1 or claim 2, wherein R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

4. A compound or pharmaceutically acceptable salt of any of claims 1 to 3, wherein R^7, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are H and R^9 is selected from (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros.

5. A compound or pharmaceutically acceptable salt of any of claims 1 to 4, wherein R^1 is H, methyl, or methoxy, for example methyl.

6. A compound or pharmaceutically acceptable salt of any of claims 1 to 5 wherein R^2 and R^6 are each independently selected from H and halogen, optionally selected from H and fluoro, for example wherein R^2 and R^6 are each H.

7. A compound or pharmaceutically acceptable salt of any of claims 1 to 6 wherein R^3, R^4 and R^5 are each independently selected from H, halogen, CN, (C1-4)alkyl and (C1-4)alkyloxy, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros, optionally wherein R^3, R^4 and R^5 are each independently selected from H, fluoro, chloro, methoxy, CN, trifluoromethyl, methoxy and trifluoromethoxy, for example.

8. A compound or pharmaceutically acceptable salt of any of claims 1 to 7 wherein R^4 is selected from H, fluoro and methoxy.

9. A compound or pharmaceutically acceptable salt of any of claims 1 to 8 wherein R^9 is selected from methyl, chloro and trifluoromethoxy, for example wherein R^9 is chloro.

10. A compound or pharmaceutically acceptable salt of any of claims 1 to 9 wherein:

    a. one of R^8 and R^{10} is H and the other is fluoro;

   b. R^8 and R^{10} are both H; or

   c. R^9 is chloro, one of R^8 and R^{10} is H and the other of R^8 and R^{10} is fluoro.

11. A compound of claim 1 selected from

   N-(3-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   N-Benzyl-2-[3-(4-chlorophenyl)-5-methoxymethyl-1*H*-1,2,4-triazol-1-yl]acetamide;

   N-Benzyl-2-[3-(4-chlorophenyl)-5-methyl-1*H*-1,2,4-triazol-1-yl]acetamide;

   N-(3-Fluorobenzyl)-2-{3-[4-(trifluoromethoxy)-phenyl]-5-methoxymethyl-1*H*-1,2,4-triazol-

   1-yl}acetamide;

   N-(3-Chlorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   N-(3-Cyanobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   N-[3-(Trifluoromethyl)benzyl]-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   N-(3-Methoxybenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   N-[3-(Trifluoromethoxy)benzyl]-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   N-(2-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   N-(4-Fluorobenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   N-(3,4-Dimethoxybenzyl)-2-[3-(4-chloro-3-fluorophenyl)-5-ethyl-1*H*-1,2,4-triazol-1-

   yl]acetamide;

   and pharmaceutically acceptable salts thereof.

12. A pharmaceutical composition comprising a compound of any of claims 1-11, or a

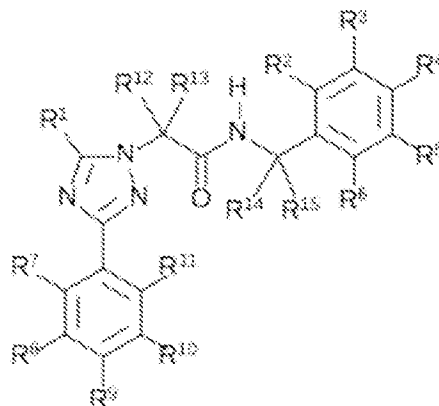   pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

13. A compound of any of claims 1 to 11, or a pharmaceutically acceptable salt thereof, for use in therapy.

14. A compound of any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, for use in the treatment or prevention of a disease or disorder mediated by excessive intracellular cyclic AMP signalling.

15. A method of treating or preventing a disease or disorder mediated by excessive intracellular cyclic AMP signalling, comprising administering to a patient in need thereof an effective amount of a compound of any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof.

16. Use of a compound of any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treatment or prevention of a disease or disorder mediated by excessive intracellular cyclic AMP signalling.

17. The compound for use, use or method of any of claims 13-16 wherein the excessive intracellular cyclic AMP signalling is caused by:

    a. excessive hormone levels produced by an adenoma.

    b. a gain-of-function gene mutation in a G-protein coupled receptor (GPCR);

    c. an activating mutation in the GNAS1 gene, which encodes the α-subunit of the G-protein G_s; or

    d. a bacterial toxin.

18. The compound for use, use or method of any of claims 13-17, wherein the disease is cancer.

19. The compound for use, use or method of claim 18, wherein the cancer is prostate cancer.

20. The compound for use, use or method of any one of claims 13-17, wherein the disease is:

    a. pituitary adenoma, Cushing's disease, polycystic kidney disease or polycystic liver disease;

       b. hyperthyroidism, Jansens's metaphyseal chondrodysplasia, hyperparathyroidism, or familial male-limited precocious puberty;

       c. McCune-Albright syndrome;

       d. cholera, whooping cough, anthrax, or tuberculosis;

       e. HIV, AIDS, or Common Variable Immunodeficiency (CVID);

       f. melanoma, pancreatic cancer, leukaemia, prostate cancer, adrenocortical tumours, testicular cancer, primary pigmented nodular adrenocortical diseases (PPNAD),or Carney Complex;

       g. autosomal dominant polycystic kidney disease (ADPKD) or autosomal recessive polycystic kidney disease (ARPKD); or

       h. maturity onset diabetes of young type 5 (MODY5); or

       i. cardiac hypertrophy.

21. The compound for use, use or method of claim 20, wherein the disease is:

       a. autosomal dominant polycystic kidney disease (ADPKD); or

       b. autosomal recessive polycystic kidney disease (ARPKD).

22. A compound for use in therapy, wherein the compound is of Formula 2:

Formula 2

wherein

R^1 is H, (C1-6)alkyl or (C3-7)cycloalkyl, the (C1-6)alkyl and (C3-7)cycloalkyl groups being optionally substituted with 1 to 3 substituents selected from OH, (C1-4)alkyloxy, (C1-4)alkylsulfonyl, C(O)-$NR^{16}R^{17}$, C(O)-OR^{16}, S(O)$_2$-$NR^{16}R^{17}$, CN and halogen;

R^2 and R^6 are independently selected from H, (C1-4)alkyl, (C1-4)alkyloxy, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^3, R^4 and R^5 are independently selected from H, (C1-4)alkyl, (C1-4)alkyloxy, (C1-4)alkylsulfonyl, C(O)-$NR^{16}R^{17}$, C(O)-OR^{16}, S(O)$_2$-$NR^{16}R^{17}$, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^7, R^8, R^{10} and R^{11} are independently selected from H and F;

R^9 is selected from H, (C1-4)alkyl, (C1-4)alkyloxy, (C1-4)alkylsulfonyl, C(O)-$NR^{16}R^{17}$, C(O)-OR^{16}, S(O)$_2$-$NR^{16}R^{17}$, CN and halogen, the (C1-4)alkyl and (C1-4)alkyloxy groups being optionally substituted with 1 to 3 fluoros;

R^{12}, R^{13}, R^{14} and R^{15} are independently selected from H and (C1-4)alkyl;

each R^{16} and R^{17}, when present, is independently selected from H and (C1-4)alkyl;

or a pharmaceutically acceptable salt thereof.

23. A compound for use according to claim 22, wherein the therapy is the treatment or prevention of a disease or disorder mediated by excessive intracellular cyclic AMP signalling.

24. A method for the treatment or prevention of a disease or disorder comprising administering to a patient in need thereof an effective amount of a compound as defined in claim 22, optionally wherein the disease or disorder is mediated by excessive intracellular cyclic AMP signalling.

25. Use of compound as defined in claim 22, in the manufacture of a medicament for the treatment or prevention of a disease or disorder mediated by abnormal excessive intracellular cyclic AMP signalling.

26. The compound for use, use or method of any one of claims 23-25, wherein the abnormal excessive intracellular cyclic AMP signalling is caused by:

    a. excessive hormone levels produced by an adenoma.

    b. a gain-of-function gene mutation in a G-protein coupled receptor (GPCR);

    c. an activating mutation in the GNAS1 gene, which encodes the α-subunit of the G-protein G_s; or

    d. a bacterial toxin.

27. The compound for use, use or method of claims 22-26, where the disease is as defined in any of claims 18 to 21.

28. A method of preparing a compound of Formula 1 as defined in claim 1 or a pharmaceutically acceptable salt thereof, comprising the step of reacting a compound of Formula 1A
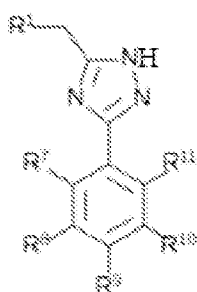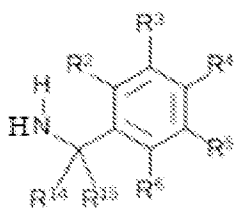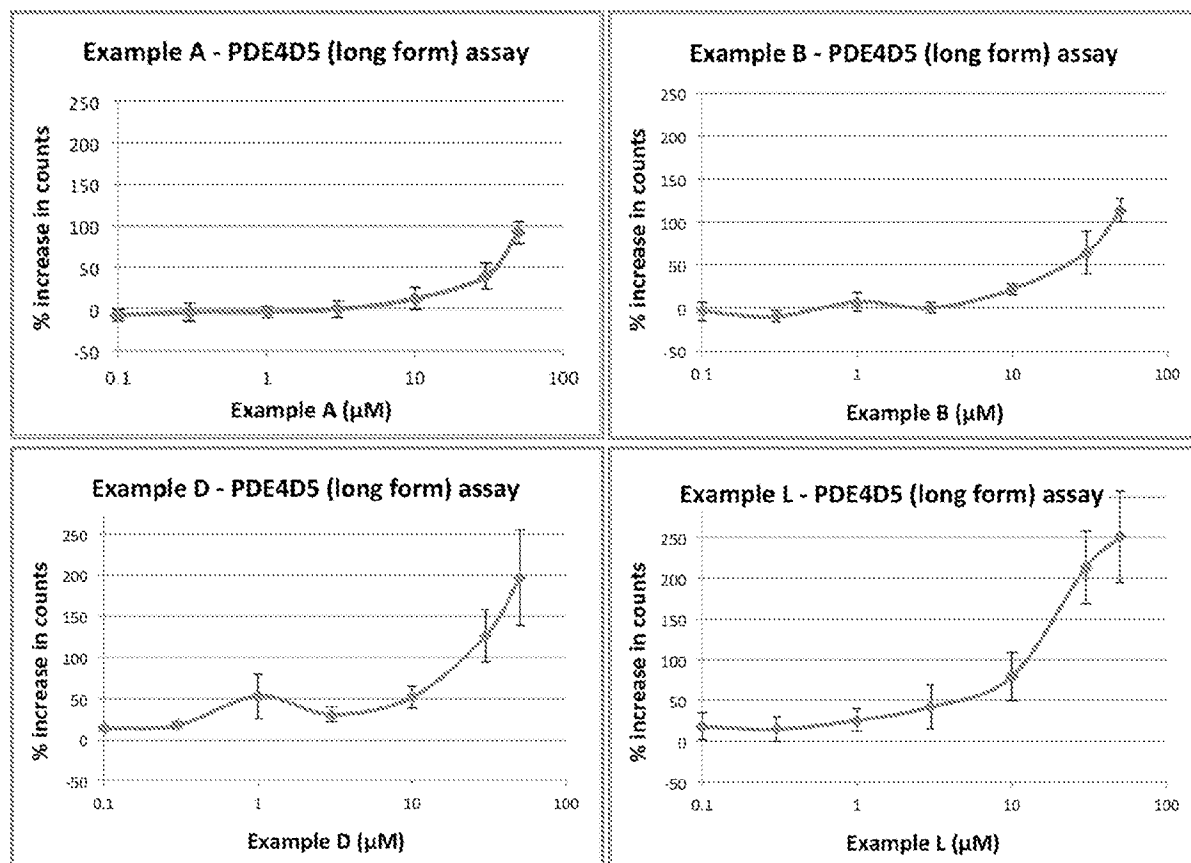

Formula 1A

with a compound of Formula 1B
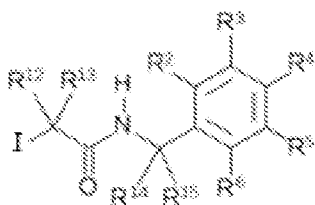
Formula1B,

wherein R^1-R^{15} are defined in claim 1.

29. A method of preparing a compound of Formula 1 as defined in claim 1 or a

pharmaceutically acceptable salt thereof, comprising the step of reacting a compound of
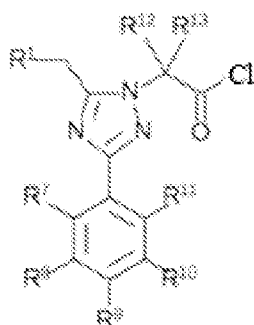
Formula 1C

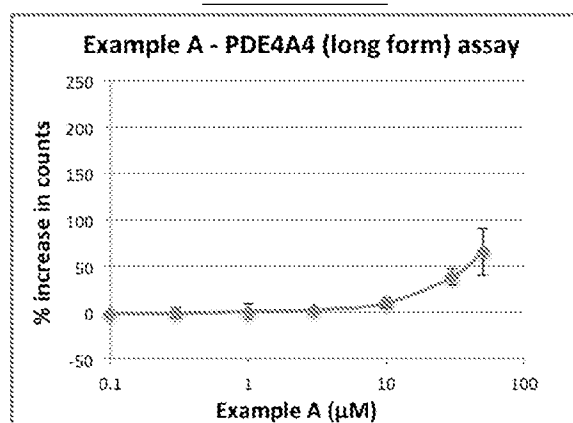Formula 1C

5   with a compound of Formula 1D

Formula 1D

wherein R^1-R^{15} are defined in claim 1.

30. A compound of Formula 1A or 1C as defined in claims 28 and 29.

31. A method of identifying a compound able to activate a long isoform PDE4 enzyme,

10   comprising the steps of:

        a. contacting a long isoform PDE4 enzyme with a candidate compound;

        b. determining whether the candidate compound activates the enzyme to at least the

            same level as a compound of Formula 1 or Formula 2.

32. A method according to claim 31, further comprising the step of:

15   c. determining whether the candidate compound does not activate a short or super-
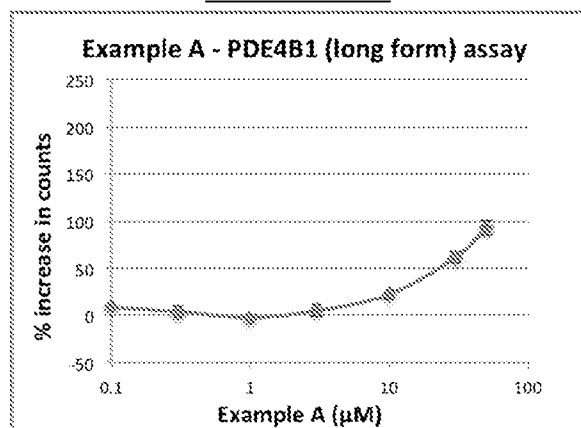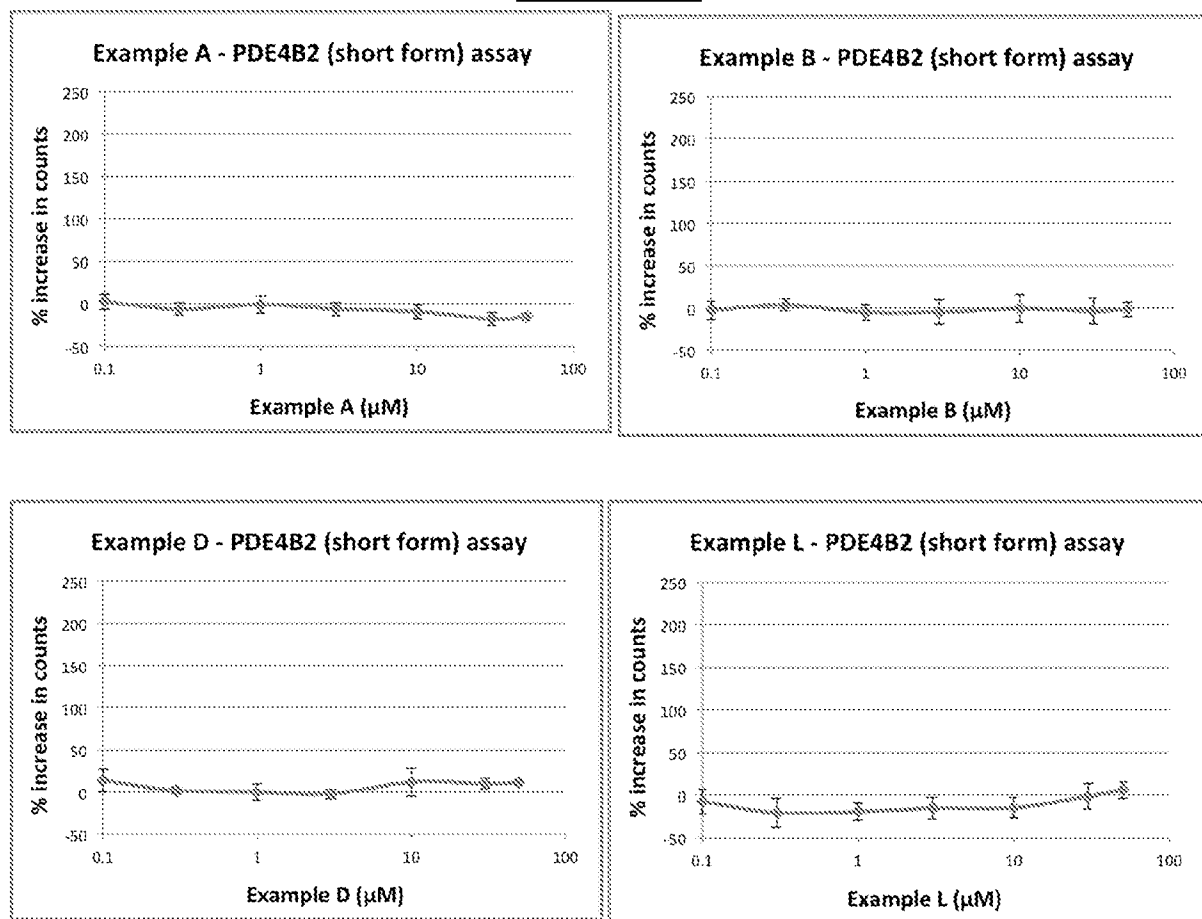
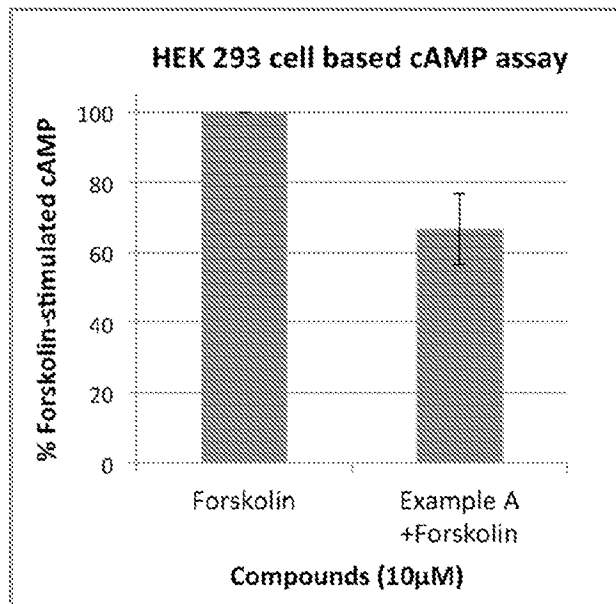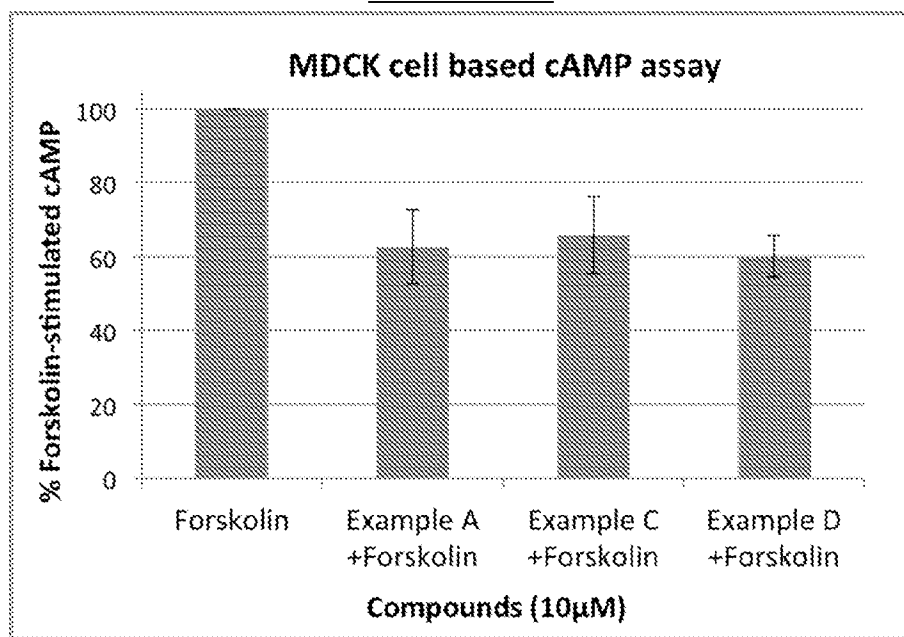   short isoform PDE4 enzyme.

FIGURE 1

Example A - PDE4D5 (long form) assay

Example B - PDE4D5 (long form) assay

Example D - PDE4D5 (long form) assay

Example L - PDE4D5 (long form) assay

FIGURE 2

Example A - PDE4A4 (long form) assay

FIGURE 3

Example A - PDE4B1 (long form) assay

FIGURE 4

Example A - PDE4B2 (short form) assay

Example B - PDE4B2 (short form) assay

Example D - PDE4B2 (short form) assay

Example L - PDE4B2 (short form) assay

FIGURE 5

HEK 293 cell based cAMP assay

FIGURE 6

MDCK cell based cAMP assay

FIGURE 7

Example A - Cyst radius at day 20
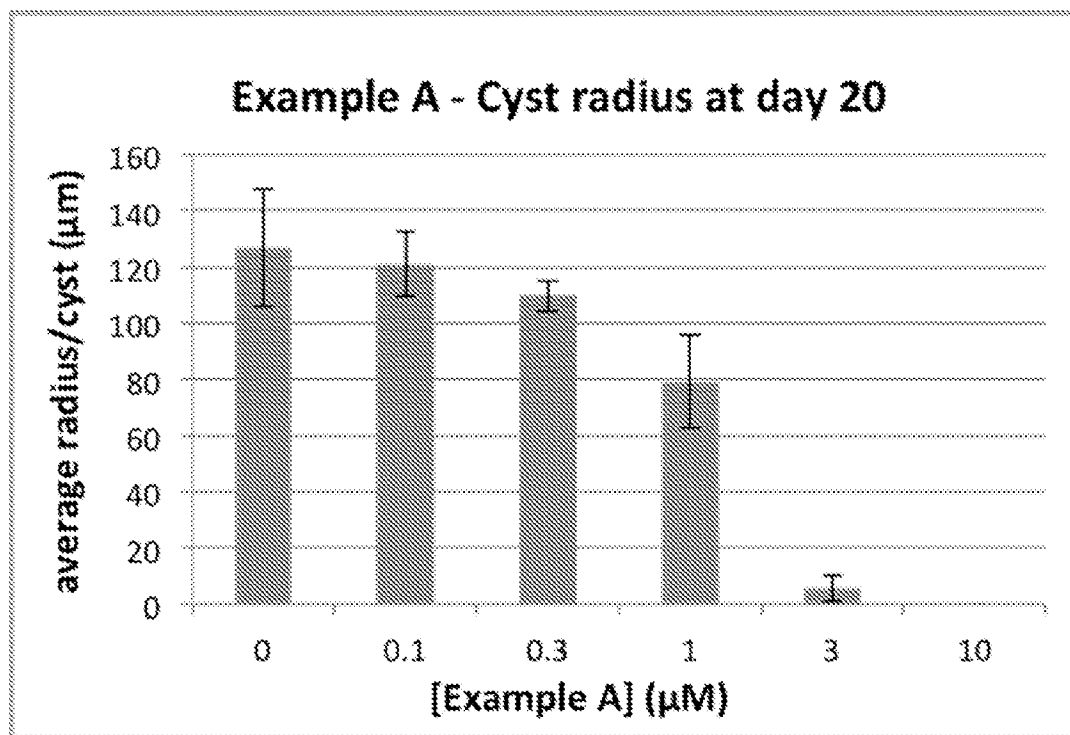
Example A – Cyst inhibition images at day 20

FIGURE 8
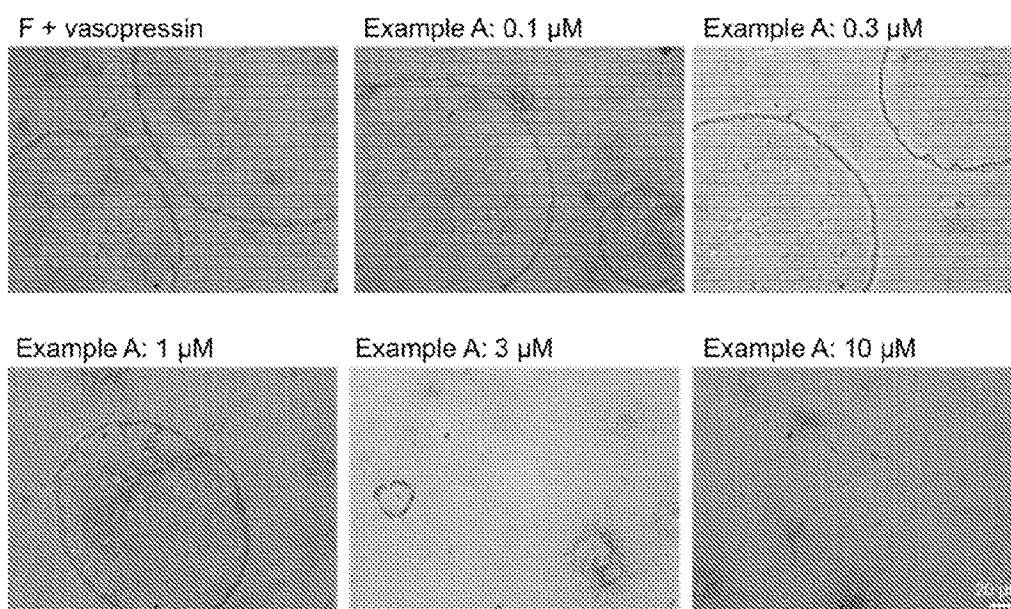
Example A - Cyst radius at day 20
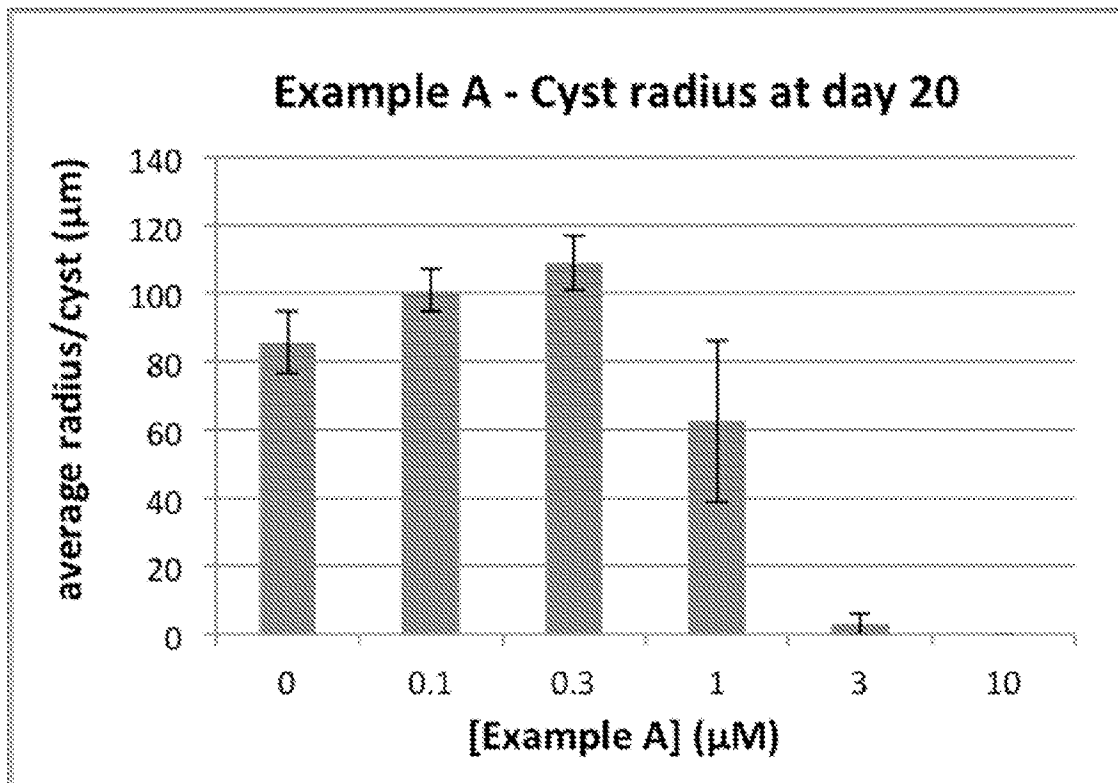
Example A – Cyst reversal images at day 20
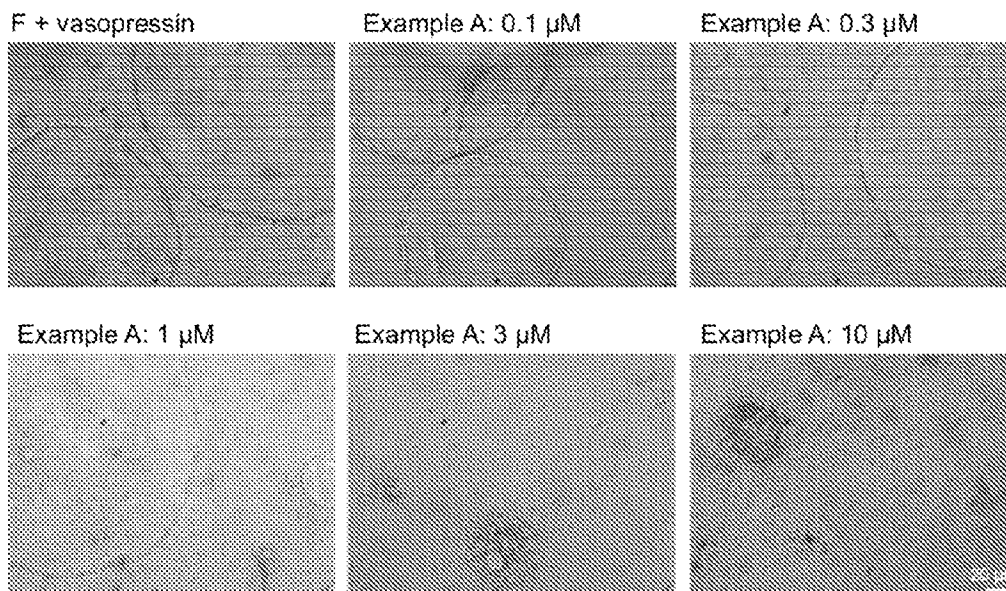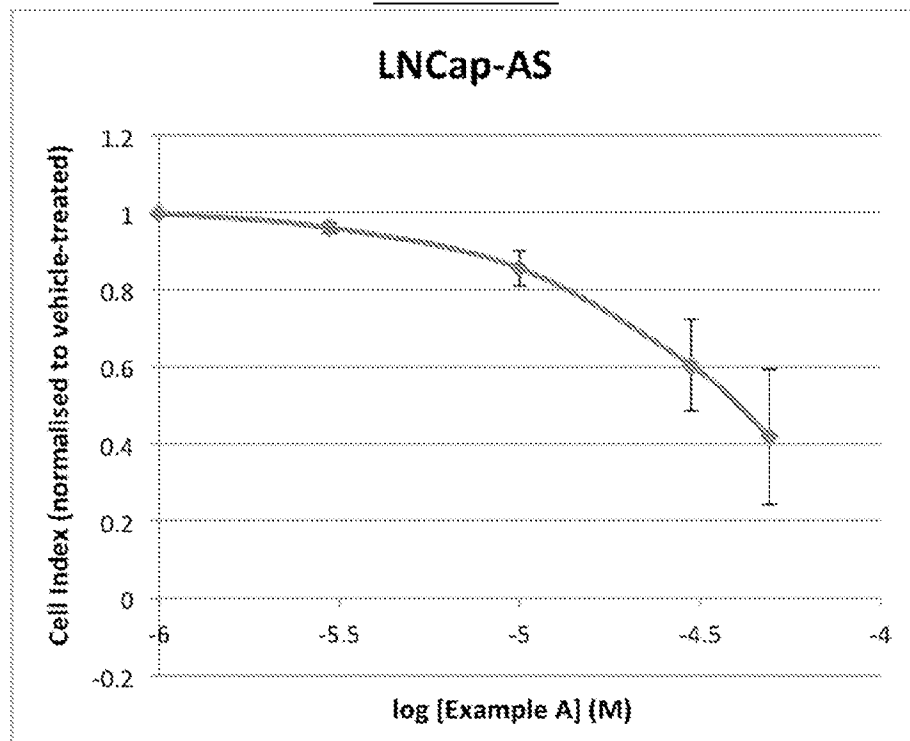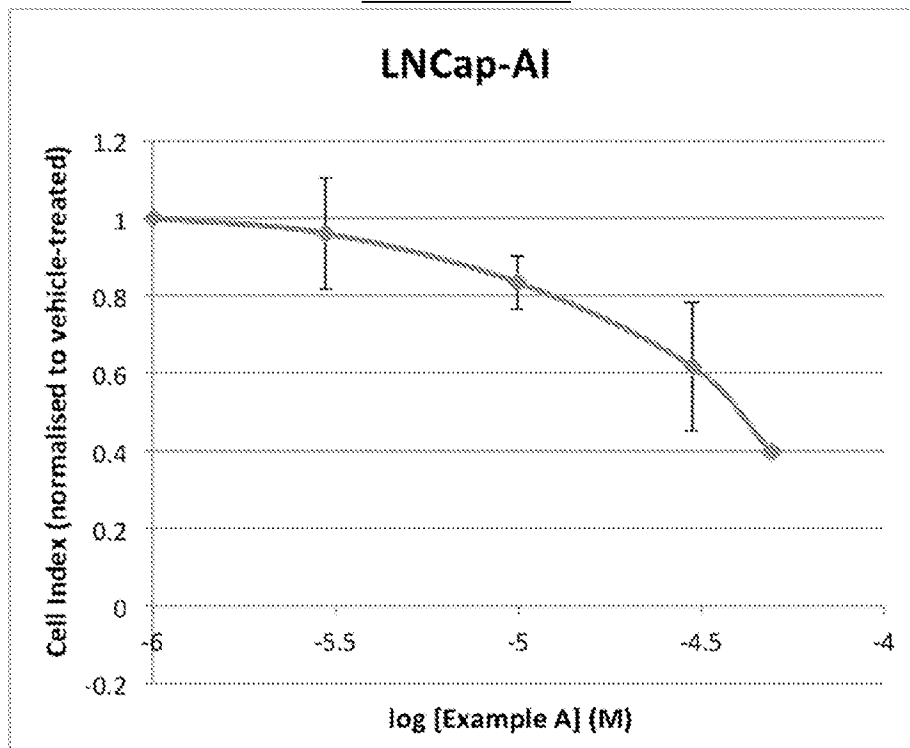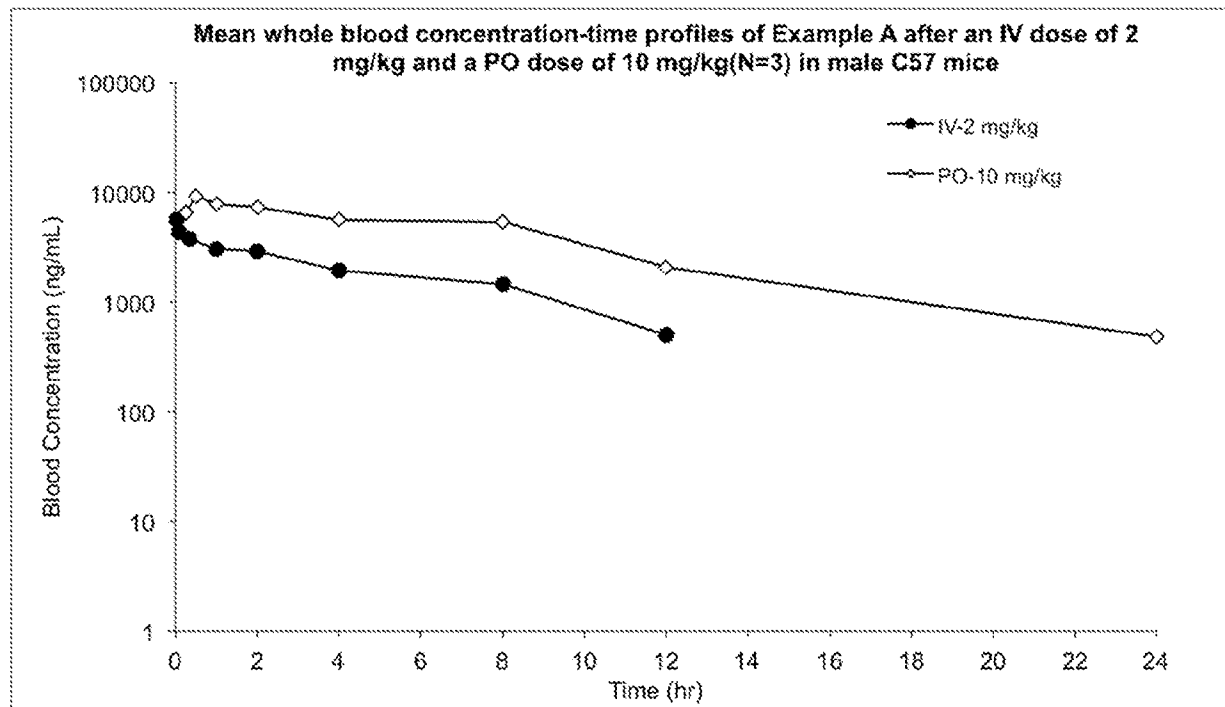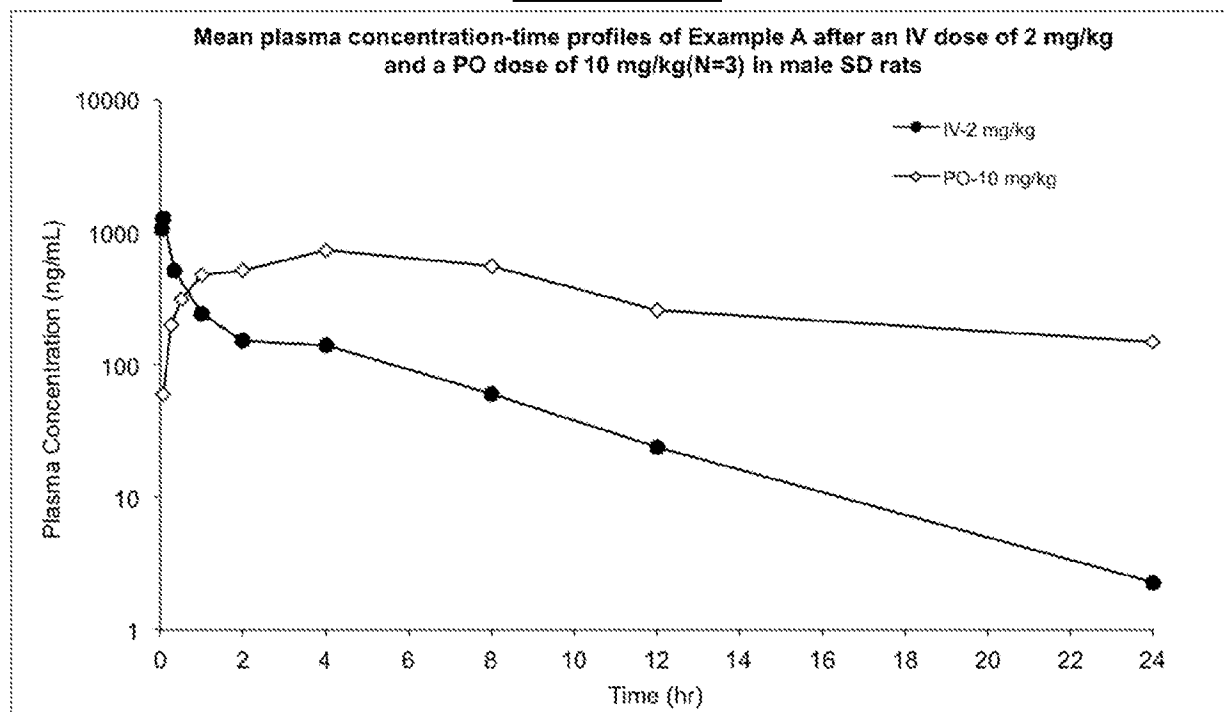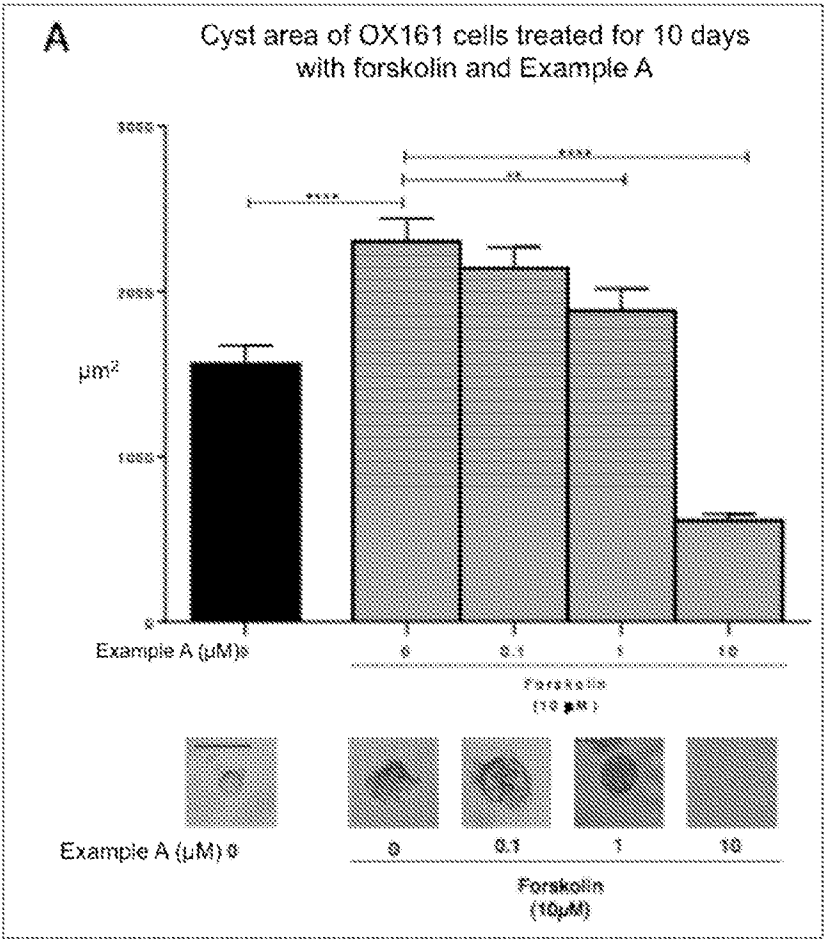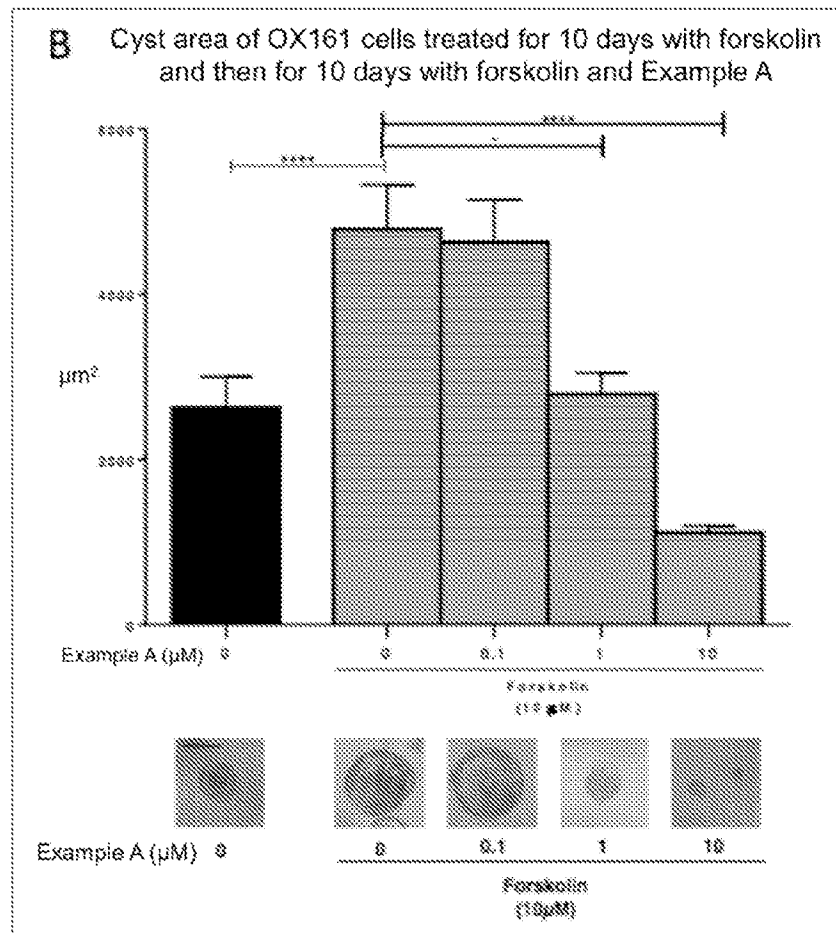
F + vasopressin   Example A: 0.1 µM   Example A: 0.3 µM

Example A: 1 µM   Example A: 3 µM   Example A: 10 µM

FIGURE 9

LNCap-AS

FIGURE 10

LNCap-AI

FIGURE 11

Mean whole blood concentration-time profiles of Example A after an IV dose of 2 mg/kg and a PO dose of 10 mg/kg(N=3) in male C57 mice

FIGURE 12

Mean plasma concentration-time profiles of Example A after an IV dose of 2 mg/kg and a PO dose of 10 mg/kg(N=3) in male SD rats

FIGURE 13

A

Cyst area of OX161 cells treated for 10 days
with forskolin and Example A

B   Cyst area of OX161 cells treated for 10 days with forskolin
     and then for 10 days with forskolin and Example A

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D249/08   A61K31/4196   A61P35/00   A61P1/00   A61P13/00
     A61P5/00   A61P11/00   A61P9/00
ADD.

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)

C07D A61P A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FRANCIS J E ET AL: "A convenient synthesis of 3,5-disubstituted-1,2,4-triazoles", TETRAHEDRON LETTERS, PERGAMON, GB, vol. 28, no. 43, 1 January 1987 (1987-01-01), pages 5133-5136, XP026649030, ISSN: 0040-4039, DOI: 10.1016/S0040-4039(00)95610-7 [retrieved on 1987-01-01] page 5134; table I; compound b -----	30
X	US 2010/222336 A1 (KONETZKI INGO [DE] ET AL) 2 September 2010 (2010-09-02) page 7, paragraphs 136, 143 page 6, paragraph 128 page 8, paragraph 151 page 9, paragraphs 171, 179, 188 -----	30

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[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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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
4 May 2016	13/05/2016

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

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/150341 A1 (GRAUERT MATTHIAS [DE] ET AL) 13 June 2013 (2013-06-13) page 62; compounds 6.02.04.27, 6.02.04.28 -----	30
X	EP 0 936 221 A1 (LEAD CHEM CO LTD [JP]) 18 August 1999 (1999-08-18) page 13; compound 5e -----	30
X	US 2013/184248 A1 (GRAUERT MATTHIAS [DE] ET AL) 18 July 2013 (2013-07-18) page 45; compounds 6.02.04.36, 6.02.04.37 -----	30
X	PETER C. WADE ET AL: "Synthesis of the triazolo[5,1-a][2,4]benzodiazepine ring system", THE JOURNAL OF ORGANIC CHEMISTRY, vol. 44, no. 1, 1 January 1979 (1979-01-01), pages 84-88, XP055270557, US ISSN: 0022-3263, DOI: 10.1021/jo01315a020 page 84; compound 10 -----	30
X	XU MENG ET AL: "An efficient and recyclable heterogeneous catalytic system for the synthesis of 1,2,4-triazoles using air as the oxidant", RSC ADVANCES: AN INTERNATIONAL JOURNAL TO FURTHER THE CHEMICAL SCIENCES, vol. 4, no. 17, 1 January 2014 (2014-01-01), page 8612, XP055270562, GB ISSN: 2046-2069, DOI: 10.1039/c3ra47029f page 8614; table 4; compounds 4f, 4g, 4h -----	30
A	US 2012/053218 A1 (BRUEGGEMEIER ULF [DE] ET AL) 1 March 2012 (2012-03-01) claims 1-11 page 9, paragraph 122-129 -----	1-32

2

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 2010222336	A1	02-09-2010	CA	2660192 A1	14-02-2008
			EP	2057152 A1	13-05-2009
			JP	2010500319 A	07-01-2010
			US	2010222336 A1	02-09-2010
			WO	2008017638 A1	14-02-2008
US 2013150341	A1	13-06-2013	EP	2788333 A1	15-10-2014
			JP	5820538 B2	24-11-2015
			JP	2015500265 A	05-01-2015
			US	2013150341 A1	13-06-2013
			WO	2013083741 A1	13-06-2013
EP 0936221	A1	18-08-1999	AU	727549 B2	14-12-2000
			AU	4318397 A	14-04-1998
			CA	2266447 A1	26-03-1998
			CN	1234032 A	03-11-1999
			CN	1346830 A	01-05-2002
			EP	0936221 A1	18-08-1999
			JP	3890453 B2	07-03-2007
			JP	H1087658 A	07-04-1998
			KR	20000036238 A	26-06-2000
			RU	2179976 C2	27-02-2002
			US	6232335 B1	15-05-2001
			US	6358984 B1	19-03-2002
			WO	9812194 A1	26-03-1998
US 2013184248	A1	18-07-2013	EP	2804854 A1	26-11-2014
			JP	5823637 B2	25-11-2015
			JP	2015506939 A	05-03-2015
			US	2013184248 A1	18-07-2013
			WO	2013107761 A1	25-07-2013
US 2012053218	A1	01-03-2012	AU	2010225241 A1	29-09-2011
			BR	PI1009240 A2	15-03-2016
			CA	2755590 A1	23-09-2010
			CN	102369190 A	07-03-2012
			DE	102009013642 A1	23-09-2010
			EP	2408754 A1	25-01-2012
			ES	2436630 T3	03-01-2014
			JP	2012520834 A	10-09-2012
			KR	20110134914 A	15-12-2011
			RU	2011141876 A	27-04-2013
			US	2012053218 A1	01-03-2012
			WO	2010105750 A1	23-09-2010