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(54) Title: NOVEL CELL-BASED PHOSPHODIESTERASE ASSAYS

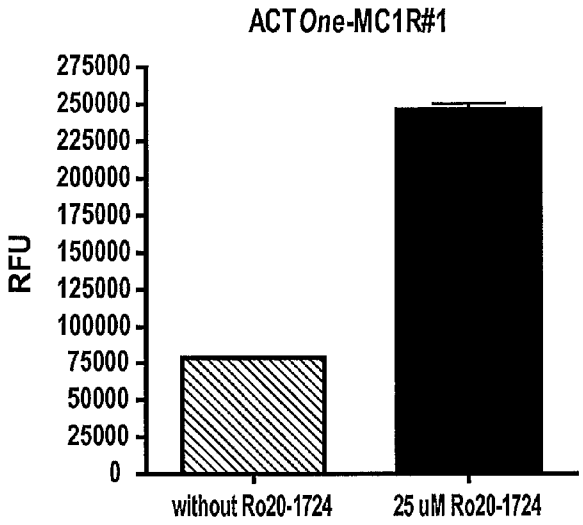

Figure 1. Fluorescence intensity of ACT*One*-MC1R#1 in the presence and absence of Ro20-1724 using ACT*One* Membrane Potential Dye

(57) **Abstract:** The present invention relates to improved cell-based assays for the in vivo assessment of phosphodiesterase (PDE) activity using cyclic nucleotide-gated channels as cyclic nucleotide sensors, and for the assessment of the effect of PDE modulating compounds.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC: **G01N 33/53**(2006.01);**C12Q 1/44**(2006.01)

USPC: 435/7.21,7.9,7.91,7.95,19
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)
U.S. : 435/7.21,7.9,7.91,7.95,19

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WUNDER et al. A cell-based cGMP assay useful for ultra-high-throughput screening and identification of modulators of the nitric oxide/cGMP pathway, Analytical Biochemistry, 26 January 2005, Vol. 339, NO. 1, pages 104-112, especially pages 106-111 and Figures 1-5.	1-4, 14, 15, 31-49, 59, 60, 74, and 75.
Y	WUNDER et al. Characterization of the First Potent and Selective PDE9 Inhibitor Using a cGMP Reporter Cell Line, Molecular Pharmacology, October 2005, Vol. 68, No. 6, pages 1775-1781, especially pages 1776-1781, Figures 1-6 and Table 1 results for PDE4B.	1-4, 14, 15, 31-49, 59, 60, 74 and 75.
Y	NEUMANN et al. Mutations in the Mouse TSH Receptor Equivalent to Human Constitutively Activating TSH Receptor Mutants Also Cause Constitutive Activity, Hormone and Metabolic Research, January 2001, Vol. 33, No. 1, pages 263-269, especially pages 264-267, Table 1 and Figures 1-4.	1-4, 14, 15, 31-49, 59, 60, 74 and 75.

☒ Further documents are listed in the continuation of Box C. ☐ 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 published on or after the international filing date

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2008 (25.08.2008)	29 SEP 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer Nashaat T. Nashed, Ph.D. Telephone No. (571) 272-1600

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RICH et al. Cyclic AMP Sensors in Living Cells: What Signals Can They Actually Measure?, Annals of Biomedical Engineering, 2002, Vol. 30, pages 1088-1099, especially pages 1090-1097 and Figures 2-6, and 9.	1-4, 14, 15, 31-49, 59, 60, 74 and 75.
Y	US 2005/0196743 A1 (OSTANIN et al) 8 September 2005 (08.09.2005), paragraphs 0015-0156 and claims 1 , 6. 9, and 11, especially paragraphs 0016-0020, 0023, 0046, 0050-0052 0050, 0059, 0060, 0066, 0077, 0079, 0080-0084, 0092, 0100, 0105-0107, 0112, 0120, 0121, and 0133.	1-4, 14, 15, 31-33, 35, 44-49, 59, 60, 74 and 75

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/04649

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

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

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1, 2, 14, 15, 31-47, 59, 60, 74 and 75, in part, and claims 3, 4, 48 and 49, particularly.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/04649

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1, 2, 14, 15, 31-33, 35-47, 59, 60, 74 and 75 drawn in part to, and claims 3, 4, 48 and 49, drawn more particularly to, a first method of identifying a phosphodiesterase (PDE) activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator, wherein the cell comprises and expresses polypeptide components of a cyclic nucleotide gated (CNG) channel .and also comprises and stably expresses an exogenous G-protein coupled receptor (GPCR), and measuring CNG channel activity by photoluminescent response to cation flux through the channel, to identify a compound capable of modulating PDE activity, whereby a measurement determining an increased CNG channel flux when a cell is contacted with a candidate modulator of CNG channel flux than in the absence of cellular contact with any compound identifies an activator of PDE activity, and a measurement determining a similar pattern of alteration of CNG.channel flux when a cell is contacted with a known PDE inhibitor and when a cell is contacted with a candidate modulator identifies a PDE inhibitor.

Group II, claims 1, 2, 14, 15, 31-33, 35-47, 59, 60, 74 and 75 drawn in part to, and claims 24, 25, 69 and 70 drawn more particularly to, a further method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and also comprises and stably expresses a GPCR mutated to increase the level of cellular cyclic nucleotide production, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PDE activator or a PDE inhibitor.

Group III, claims 1, 2, 14, 15, 31-33, 35-47, 59, 60, 74 and 75 drawn in part to, and claims 5, 6, 50, and 51 drawn more particularly to, another method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and also comprises and stably expresses an exogenous G-protein, including a Gas, Gαolf, mutant, variant, or chimeric G-protein having no particular alteration of its activity, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PD.E activator or a PDE inhibitor.

Group IV, claims 1, 2, 14, 15, 31-33, 35-47, 59, 60, 74 and 75 drawn in part to, and claims 24, 25, 69, and 70 drawn more particularly to, another method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and also comprises and stably expresses a G-protein mutated to increase the level of cellular cyclic nucleotide production, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PDE activator or a PDE inhibitor.

Group V, claims 1, 2, 14, 15, 31-33, 35-47, 59, 60, 74 and 75 drawn in part to, and claims 7, 8, 52 and 53 drawn more particularly to, another method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and also comprises and stably expresses an exogenous adenylate cyclase, including a mutant, variant, or chimeric adenylate cyclase having no particular alteration of its activity, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PDE activator or a PDE inhibitor.

Group VI, claims 1, 2, 14, 15, 31-33, 35-47, 59, 60, 74 and 75 drawn in part to, and claims 24, 25, 69, and 70 drawn more particularly to, another method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and also comprises and stably expresses an adenylate cyclase mutated to increase the level of cellular cyclic nucleotide production, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PDE activator or a PDE inhibitor.

Group VII, claims 1, 2, 14-16, 31-33, 35-67, 59, 60, 61, 74 and 75 drawn in part to, and claims 17-19 and 62-64 drawn more particularly to, another method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and wherein the expression of an endogenous protein capable of affecting PDE activity is mediated by a constitutive promoter, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PDE activator or a PDE inhibitor.

Group VIII, claims 1, 2, 14-16, 31-33, 35-46, 59, 60, 61, 74 and 75 drawn in part to, and claims 20-23 and 65-68, drawn more particularly to, another method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and wherein the expression of an endogenous protein capable of affecting PDE activity is mediated by an inducible promoter, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PDE activator or a PDE inhibitor.

Group IX, claims 1, 26, 31-33, 35-46, 54, 74 and 75 drawn in part to, and claims 9, 11-13, 27-30, and 71-73 drawn more particularly to, another method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and also comprises and expresses an exogenous, unmodified, PDE, which may be a PDE4, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PDE activator or a PDE inhibitor.

Group X, claims 1, 26, 31-33, 35-46, 54, 74 and 75 drawn in part to, and claims 10 and 55-58 drawn more particularly to, another method of identifying a PDE activity modulator, and cells used in the method, by contacting a mammalian cell, such as a HEK-293 cell, with a candidate modulator wherein the cell comprises and expresses polypeptide components of a CNG channel and also comprises and expresses an exogenous mutant, variant, or chimeric PDE, whereby measurements determining an increased CNG channel flux, or a similar pattern of alteration of CNG channel flux, when a cell is contacted with a candidate modulator identify, respectively, a PDE activator or a PDE inhibitor.

Group XI, claims 76-80, drawn to an independent product, a kit comprising cells, such as HEK-293 cells, comprising and expressing polypeptide components of a CNG channel and further comprising and expressing an exogenous polypeptide that increases cyclic nucleotide production without an external stimulation of cyclic nucleotide production, as well as comprising one or more reagents, and, optionally, at least one indicator.

The method of Group I lacks unity of invention with methods of the inventions of Groups II-XI because the generic method described by claims 1, 2, 14, 31-33, 35-47, 59 and 60, and particularly including a method of claims 3 and 48 lacks a technical feature that is special where the method is disclosed in the prior art publication of WO 2004/083803, in, e.g., claims 1-11 and 15 wherein the modulation of ion flow through a CNG channel is measured upon contact with a GPCR by a "test compound " such as a ligand of claim 8, where claim 9 requires expression of an exogenous GPCR in accord with claims 1-3 and 44-48 of this application and where an ultimate modulation of ion flow by (a) ligand(s) through a CNG channel renders them a significant subset of candidate "compounds" of claims 1-3 and 44-48 herein. This is also because teachings at paragraphs 0092, 0105, 0106, 0120, and the portion of paragraph 0121 spanning the mid-left column through the right column of page 13 of the prior art publication of Ostanin et al. show that the mammalian cells transfected for subsequent exposure to candidate compounds when seeded in 384-well and 1456-well microtiter plates at page 4, lines.19-31, of WO 2004/083803, may be a HeLa cell, CHO cell, BHK cell or HEK-293 cell that stably expresses an exogenous unaltered GPCR or a mutant GPCR. The method Group I therefore shares no same or corresponding technical feature that is special with methods of Groups II-XI where claims of Group I provide no contribution that distinguishes them over disclosures of the prior art.

The method of Group II lacks unity of invention with the methods and product of the inventions of Groups III-XI because the special technical feature of the invention of Group II is the cellular expression of a GPCR that is mutated to increase cellular cyclic nucleotide levels, which expression may be mediated by an endogenous promoter, while the technical features of the methods of Groups II-XI do not require cellular expression of an exogenous GPCR that is mutated to increase cellular cyclic nucleotide levels. Thus the invention of Group II shares no same or corresponding technical feature with the inventions of Groups III-XI.

The method of Group III lacks unity of invention with the methods and product of the inventions of Groups IV-XI because the special technical feature of the invention of Group IV is the cellular expression of an exogenous G protein having no particular alteration of its activity, which expression may be mediated by an endogenous promoter, while the technical features of the methods of Groups IV-XI either require a particular modification of the activity of an expressed G protein or do not particularly require cellular expression of an

exogenous G protein. Thus the invention of Group III shares no same or corresponding technical feature with the inventions of Groups IV-XI.

The method of Group IV lacks unity of invention with the methods and product of the inventions of Groups V-XI because the special technical feature of the invention of Group IV is the cellular expression of a G protein that is mutated to increase cellular cyclic nucleotide levels, which expression may be mediated by an endogenous promoter, while the technical features of the methods of Groups V-XI do not require the cellular expression of a G protein that is mutated to increase cellular cyclic nucleotide levels. Thus the invention of Group IV shares no same or corresponding technical feature with the inventions of Groups V-XI.

The method of Group V lacks unity of invention with the methods and product of the inventions of Groups VI-XI because the special technical feature of the invention of Group V is the cellular expression of an exogenous adenylate cyclase having no particular alteration of its activity, which expression may be mediated by an endogenous promoter, while the technical features of the methods of Groups VI-XI either require the cellular expression of an adenylate cyclase mutated to increase cellular cyclic nucleotide levels or do not particularly require the cellular expression of an adenylate cyclase. Thus the invention of Group V shares no same or corresponding technical feature with the inventions of Groups VI-XI.

The method of Group VI lacks unity of invention with the methods and product of the inventions of Groups VII-XI because the special technical feature of the invention of Group IV is the cellular expression of an adenylate cyclase mutated to increase cellular cyclic nucleotide levels, Which expression may be mediated by an endogenous promoter, while the technical features of the methods of Groups VII-XI do not require the cellular expression of an adenylate cyclase mutated to increase cellular cyclic nucleotide levels. Thus the invention of Group IV shares no same or corresponding technical feature with the inventions of Groups VII-XI.

The method of Group VII lacks unity of invention with the methods and product of the inventions of Groups VIII-XI because the special technical feature of the invention of Group VII is the cellular expression of an exogenous polypeptide that is mediated by an heterologous, constitutive, promoter, while the technical features of the methods of Groups VIII-XI do not require that the cellular expression of an exogenous polypeptide be mediated by an heterologous constitutive promoter. Thus the invention of Group VII shares no same or corresponding technical feature with the inventions of Groups VIII-XI.

The method of Group VIII lacks unity of invention with the methods and product of the inventions of Groups IX-XI because the special technical feature of the invention of Group VIII is the cellular expression of an exogenous polypeptide that is mediated by an inducible promoter, while the technical features of the methods of Groups IX-XI do not require that the cellular expression of an exogenous polypeptide be mediated by an inducible promoter. Thus the invention of Group VIII shares no same or corresponding technical feature with the inventions of Groups IX-XI.

The method of Group IX lacks unity of invention with the methods and product of the inventions of Groups X and XI because the special technical feature of the invention of Group IX is the cellular expression of an exogenous an exogenous unmodified PDE, which may be a PDE4, while the technical features of the methods of Groups X and X1 do not either require that the cellular expression of an exogenous mutant, variant, or chimeric PDE or do not require the expression of an exogenous PDE. Thus the invention of Group IX shares no same or corresponding technical feature with the inventions of Groups X and XI.

The method of Group X lacks unity of invention with the product of the invention of Group XI because the special technical feature of the invention of Group IX is the cellular expression of an exogenous mutant, variant, or chimeric PDE, while the product of Group XI does not require the presence in, or expression by, a cell of an exogenous mutant, variant, or chimeric PDE. Thus the invention of Group X shares no same or corresponding technical feature with the invention of Group XI.