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Señor Director
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Superintendencia de Industria y Comercio
E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-086605- -00003-0000

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Trámite : 011
Evento : 378
Actuación : 444

Referencia : Expediente No. **07.086.605**

Asunto : Solicitud de patente para: **NUEVOS MEDICAMENTOS PARA EL TRATAMIENTO DE LAS ENFERMEDADES DE LAS VIAS RESPIRATORIAS**

Solicitante : **BOEHRINGER INGELHEIM INTERNATIONAL GMBH**

Fecha solicitud: 23 de agosto de 2007

1. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, me refiero al oficio No. **2327** notificado por fijación en lista del 11 de febrero de 2011.

2. Mediante oficio No. **2327** se puso en conocimiento del solicitante el informe que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente. Las observaciones realizadas en el oficio son las siguientes:

2.1 De la solicitud de Patente

En forma resumida el Examinador afirma que las reivindicaciones 1 a 12 no son patentables, por cuanto hacen referencia a un uso.

2.2 Análisis de Patentabilidad

2.2.1 Novedad

Afirma el Examinador que las características esenciales de la reivindicación 13 ya habían sido anticipadas en D1 y que por lo tanto, la presente invención no es nueva.

3. Respuesta a las objeciones

De este modo y con el objeto de atender las objeciones arriba mencionadas, se desean poner de presente las siguientes modificaciones, aclaraciones y argumentos:

3.1 Modificación de una solicitud en trámite y aclaraciones

De acuerdo con el artículo 34 de la Decisión 486 de la Comisión de la Comunidad Andina, donde se establece que la modificación de una solicitud es posible en cualquier momento del trámite mientras no implique una ampliación de la solicitud inicial, me permito presentar un nuevo capítulo descriptivo y un nuevo pliego reivindicatorio conformado por 7 reivindicaciones.

Inicialmente, deseamos aclarar que de acuerdo con las observaciones hechas por el Examinador, las reivindicaciones 1 a 12 no han sido incluidas en el nuevo pliego reivindicatorio. De esta manera las anteriores reivindicaciones 13 a 19 han sido reenumeradas con el fin de guardar correspondencia. Adicionalmente, las limitaciones de la anterior reivindicación 14 han sido incluidas en la nueva reivindicación 1. Igualmente, la anterior reivindicación 15 ha sido modificada.

Solicitamos atentamente tener en cuenta este nuevo pliego reivindicatorio, el cual no amplía el alcance de la invención y cumple con las disposiciones de la Decisión 486.

Finalmente deseamos poner de presente, que a pesar de que en el nuevo pliego reivindicatorio no se incluyó materia inicialmente reivindicada, mi representada no renuncia a la protección que pueda obtener sobre dicha materia y se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud, beneficiándose de la fecha de prioridad inicialmente reivindicada.

3.2 Argumentos a favor de la novedad de la solicitud

En concordancia con el Artículo 16 de la Decisión 486, una invención se considera novedosa cuando no está comprendida en el estado de la técnica. Lo anterior indica que

toda invención debe ofrecer al menos una característica técnica nueva sobre el estado del arte más cercano.

A este respecto y considerando que el Examinador objeta la falta de novedad de la presente invención frente a D1, deseamos poner de presente que de acuerdo con la materia revelada por el actual pliego reivindicatorio, se debe tener por superada la objeción de novedad, debido a que el documento D1 no revela compuestos como los de la presente solicitud.

Respetuosamente discrepamos de la opinión del Examinador y a este respecto, se aclara al Despacho que *El Manual Para El Examen De Solicitudes De Patentes de la Comunidad Andina*¹, que en muchos aspectos coincide con las Directrices de Examen de la Oficina Europea de Patentes (Guidelines for Examination in the EPO), y el cual corresponde a un documento que tiene por objeto la armonización de los procedimientos y prácticas en materia de patentes además de servir como elemento de trabajo para los examinadores de las Oficinas nacionales de patentes, ha sostenido lo siguiente:

"Una formula general no destruye la novedad de un compuesto o un subgrupo de compuestos incluidos en ella. Compuestos específicos en un documento destruyen la novedad de una fórmula general" (Subrayado fuera de texto).

"Si una fórmula tiene sustituyentes específicos listados, se considera que la selección de una de las posibilidades cuando sólo hay una lista de alternativas para un sustituyente carece de novedad. Es decir, se considera una fórmula general con variación en un solo sustituyente y en el que todas las alternativas para este sustituyente estén listadas es equivalente al listado de todos los compuestos específicos. Sin embargo, si hay que hacer una selección en dos listas o más de substituyentes para llegar al

¹ IEPI, BID. "MANUAL PARA EL EXAMEN DE SOLICITUDES DE PATENTES DE INVENCION EN LAS OFICINAS DE PROPIEDAD INDUSTRIAL DE LOS PAISES DE LA COMUNIDAD ANDINA". Quito, Ecuador 2004. Pág. 65.

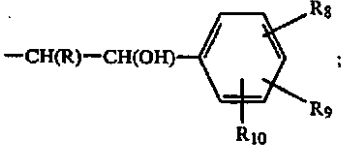
objeto de la reivindicación, entonces se considera que hay
novedad" (Subrayado fuera de texto).

Por otro lado, según las Directrices de Examen de la Oficina Europea de Patentes, (C-IV-
9.8), en el caso de solapamiento de fórmulas químicas existe novedad si un grupo
químico seleccionado específicamente, aún estando descrito de forma general en el
estado de la técnica, no está individualizado en el documento del estado de la técnica.

En la presente invención se cumplen las dos situaciones mencionadas en los párrafos
anteriores:

1) La definición de los sustituyentes de los compuestos de la presente invención está
dada por una selección entre las diferentes listas (más de una) de sustituyentes descritas
en D1, tal y como se indica a continuación:

	D1	Invención
R1 y R2	Hidrógeno o alquilo de 1 a 4 átomos de carbono	Etilo o propilo o en conjunto -CH ₂ -CH ₂ -
R3 y R4	hidrógeno, flúor, cloro, hidroxilo, metilo, etilo ó alcoxi de 1 a 4 átomos de carbono	R3=flúor R4=hidrógeno
R5 y R6	Hidrógeno ó metilo	metilo

	D1	Invención
R7		
R	Hidrógeno ó alquilo de 1 a 4 átomos de carbono	hidrógeno
R8	Flúor, cloro, alquilo de 1 a 4 átomos de carbono, (alquilo de 1 a 4 átomos de carbono)tio, -CONHR ₁₁ , -SO ₂ NHR ₁₁ , -OR ₁₂ , metoxicarbonilo, etoxicarbonilo ó -SO ₂ NHCH ₃	-SO ₂ NHCH ₃
R9	Hidrógeno, flúor, cloro, ó -OR ₁₂	-OR ₁₂
R10	Hidrógeno, clóro, amino, metilo, ó metoxi	hidrógeno
R11	Hidrógeno, metilo , etilo ó hidroximetilo	metilo
R12	Hidrógeno, alquilo de 1 a 4 átomos de carbono entre otros	hidrógeno
n	1, 2 ó 3	1

2) aunque los sustituyente R1 y R2 de los compuestos de fórmula I descritos en D1 pueda, de forma genérica, representar grupos etilo y propilo, no hay ningún ejemplo específico en D1 donde R1 y R2 representen estos grupos o que tenga el anillo ciclopropílico y que, simultáneamente, presente las definiciones de R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, R₁₂ y n de acuerdo con los significados de la misma según la presente invención. El hecho que se mencionen de manera general algunos significados de los sustituyentes de los compuestos de la fórmula 1 de la presente invención, no hace que dichos significados en conjunto se encuentren específicamente ejemplificados.

Por lo tanto, siguiendo las indicaciones de la Oficina Europea de patentes que recoge el Manual Andino de Patentes, las reivindicaciones de la presente invención no pueden ser objetadas por falta de novedad frente a D1.

En efecto, solicitamos que a partir de la interpretación que a esta norma le ha dado el Manual Andino de Patentes se evalúe la patentabilidad de la presente invención. Para estos efectos se debe tener en cuenta que si bien existe una superposición entre los compuestos generales de formula I de D1 y los compuestos descritos en la presente solicitud, dicha anterioridad no describe ni sugiere en sus ejemplos o específicamente, el hecho de elegir y combinar ciertos radicales de las listas de sustituyentes dadas en la formula general I de D1, con el fin de obtener los compuestos específicos de la presente invención.

En consecuencia, partiendo de las enseñanzas de D1, la presente solicitud debe ser considerada como una invención que no resulta obvia, debido a que dicha selección se realiza de diferentes listas de sustituyentes.

Así las cosas, los compuestos de la presente invención se debe considerar como una invención de selección por cuanto la materia reivindicada es producto de la selección entre las diferentes listas de sustituyentes descritas en D1, toda vez que para obtener los mismos, se debe realizar una selección múltiple de al menos las listas de R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, R₁₂ y n de D1, siendo, en consecuencia, los compuestos de la presente solicitud, novedosos frente a las enseñanzas de D1.

Por lo tanto, siendo claro que el objeto de la anterioridad citada y el de la presente invención es diferente no es posible concluir que la primera sea una anterioridad válida capaz de afectar la novedad de ésta última y por ende, se puede concluir que los compuestos acá reivindicados son novedosos a la luz de D1.

De acuerdo con lo anterior, solicitamos amablemente al Examinador que reconsidere y tenga por superada la objeción de novedad formulada, por cuanto la anterioridad citada no revela todas las características técnicas de los compuestos reivindicados y consecuentemente no puede ser considerado una anterioridad válida capaz de afectar la novedad de la presente solicitud.

Además, deseamos allegar a este memorial datos adicionales (Documento: "Arguments and Supporting Data") en los cuales se muestra la superioridad de los compuestos de la presente invención.

En vista de lo anterior, consideramos la presente invención no solo es novedosa frente a D1 sino que también posee nivel inventivo.

Puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, respetuosamente solicito se continúe con su tramitación. Sin embargo, en el evento que el Despacho considere que una modificación adicional es necesaria, respetuosamente le solicito hacer uso de la facultad que le otorga el artículo 45 inciso 2 de la Decisión 486 de la Comisión de la Comunidad Andina a efecto de requerir nuevamente de nuestra parte cualquier complementación o aclaración.

4. ANEXOS

- Nuevo pliego reivindicatorio conformado por 7 reivindicaciones
- Formulario de modificaciones.
- Documento: "Arguments and Supporting Data"
- Copia auténtica la sustitución del poder otorgado por el Doctor EMILIO FERRERO, a mi favor, para lo cual solicito me sea reconocida personería y así continuar con el trámite de este asunto en favor de la sociedad solicitante.

Señor Director,


LUZ CLEMENCIA DE PÁEZ
C.C. 35.456.344 de Usaquén
T.P.A. No. 23.555

FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES

1	SOLICITUD DE: ☐ Corrección de Error Material ☒ Modificación a una solicitud	
2 SOLICITANTE	Nombre: BOEHRINGER INGELHEIM INTERNATIONAL GMBH Sociedad constituida y existente bajo las leyes de Alemania. Dirección: 55216 Ingelheim am Rhein, Alemania Domicilio: Ingelheim am Rhein, Alemania Teléfono: 546 09 70 Fax: 249 40 70 E-mail: clientes@cavelier.com	IDENTIFICACION C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Número: _____
3 REPRESENTANTE O APODERADO	Nombre: Luz Clemencia de PAEZ Dirección: Carrera 4ª No. 72-35 Teléfono: 347 36 11 Fax: 217 92 11 E-mail: cavelier@cavelier.com	IDENTIFICACION C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Número: <u>35.456.344</u> TP <u>23.555</u>
4	Número de expediente: <u>07.086.605</u>	
5	Descripción de la corrección o modificación solicitada: Presento nuevo capítulo reivindicatorio compuesto por 7 reivindicaciones con el fin de superar las objeciones hechas por Su Despacho a la presente solicitud de patente y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas. _____ _____	
6	Comprobante de pago No. _____ Fecha: _____	
7	Anexos ☐ Comprobante de pago de la tasa por \$118.000, por concepto de modificaciones. ☐ Comprobante de pago por reivindicaciones adicionales después de la décima reivindicación por. ☐ Poderes, si fuere el caso ☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas. ☒ Copia auténtica de la sustitución que del poder otorgado por la Sociedad Solicitante hizo el Doctor EMILIO FERRERO a mi favor, y para lo cual solicito me sea reconocida personería para continuar con el trámite del asunto de la referencia.	

8

NOMBRE: LUZ CLEMENCIA DE PAEZ FIRMA: Luz Clemencia De Paez

C.C. 35.456.344 de Usaquén T.P.A. 23.555

DVF

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Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E. S. D.

Poderdante : BOEHRINGER INGELHEIM INTERNATIONAL GMBH

Poder conferido el : 12/12/1995

Yo, EMILIO FERRERO, obrando como apoderado principal del poderdante arriba mencionado, mediante el presente escrito sustituyo el poder a mi otorgado a la doctora LUZ CLEMENCIA DE PAEZ, identificada con cédula de ciudadanía No. 35.456.344 y Tarjeta Profesional N°23.555, con todas las facultades que me fueron conferidas.

Hago la anterior sustitución conforme al artículo 66 del Código de Procedimiento Civil.

Señora Jefe,

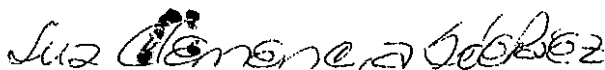


EMILIO FERRERO

T.P.A. N° 31.929

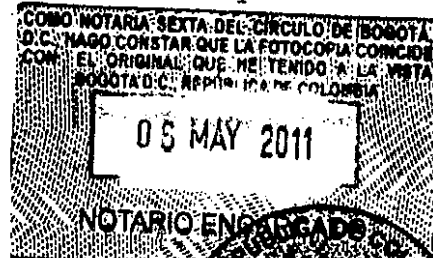
C.C. N° 438.019 de Usaquén

Acepto:


LUZ CLEMENCIA DE PAEZ

T.P.A. N° 23.555

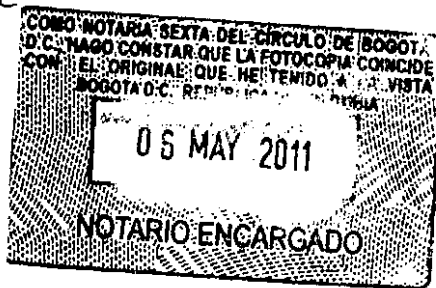
C.C. No. 35.456.344 de Usaquén



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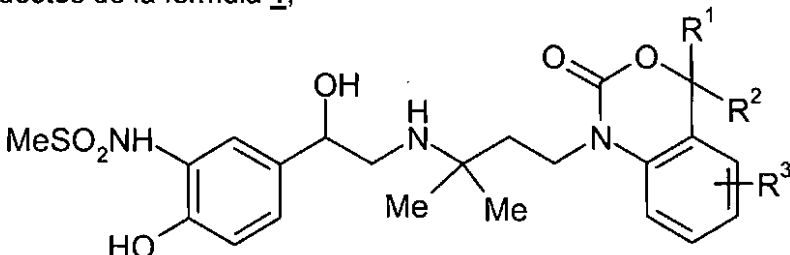
NOTARIA SEXTA DE BOGOTA
RECONOCIMIENTO Y PRESENTACION PERSONAL
Bogotá, D.C.
Ante el **03 NOV 2009**
NOTARIO SEXTO (E) DEL CIRCULO DE
BOGOTA D.C.
Compareció(eron) *Quinto Pineda*
Para Celerencia de
...
Quien(es) exhibió(aron) la(s) C.C.
C.C. 7458019-1-142
C.C. 3545684-7-2335
Y declaró(aron) que la(s) firma(s) que aparece(n)
en el presente documento es(son) la(s) suya(s) y
que el contenido del mismo es cierto.
En constancia se firma esta diligencia.

Emili G...
Luz Clemencia Gótz



REIVINDICACIONES

1. Compuestos de la fórmula 1,

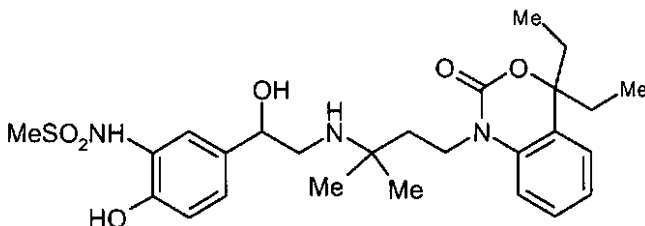


en la cual significan

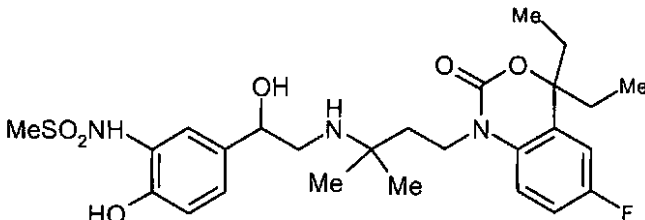
R¹ y R² ambos representan etilo o propilo o en conjunto representan -CH₂-CH₂-, y

R^3 representa hidrógeno ó flúor.

2. Compuesto de la fórmula **1** de acuerdo con la reivindicación 1, el cual corresponde a N-(5-{2-[3-(4,4-dietil-2-oxo-4H-benzo[d][1,3]oxazin-1-il)-1,1-dimetil-propilamino]-1-hidroxi-etil}-2-hidroxi-fenil)-metanosulfonamida **1a**.



3. Compuesto de la fórmula **1** de acuerdo con la reivindicación 1, el cual corresponde a N-(5-[2-[3-(4,4-dietil-6-fluor-2-oxo-4H-benzo[d][1,3]oxazin-1-il)-1,1-dimetil-propilamino]-1-hidroxi-etil]-2-hidroxi-fenil)-metanosulfonamida **1b**.



4. Compuestos de la fórmula general 1 de acuerdo con una de las reivindicaciones 1 a 3, en donde los compuestos de la fórmula 1 se presentan en la forma de los isómeros ópticos individuales, mezclas de los enantiómeros individuales eventualmente

diastereómeros o racematos, preferentemente en la forma de los compuestos enantioméricamente puros eventualmente diastereoméricamente puros.

5. Compuestos de la fórmula general 1 de acuerdo con una de las reivindicaciones 1 a 3, en donde los compuestos de la fórmula 1 se presentan en la forma de las bases libres o en la forma de las sales de adición de ácido con ácidos farmacológicamente inocuos así como eventualmente en la forma de los solvatos y / o hidratos.

6. Compuestos de la general 1 de acuerdo con una de las reivindicaciones 1 a 5 como composiciones farmacéuticas.

7. Formulación farmacéutica, caracterizada por un contenido de un compuesto de la fórmula 1 de acuerdo con una de las reivindicaciones 1 a 5.

Arguments and Supporting data

Long- and short-acting betamimetics are used due to their bronchodilatory effect for the treatment of pulmonary diseases and thus are proposed by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as a controller and reliever medication for the treatment of chronic obstructive pulmonary disease (COPD; see GOLD pocket guide 2008 under goldcopd.com, pages 18 and 21).

To avoid unwanted side effects, in particular cardiovascular side effects (e.g. tachycardia, other side effects are hypokalemia and muscle tremor) it is important, that the used betamimetic is as inactive as possible on the β_1 adrenoceptor. Therefore, such betamimetics are most suitable, which are characterized in the used functional assay either

- i) by a low intrinsic activity on the β_1 adrenoceptor,
- ii) by a high selectivity (calculated from the ratio of the EC_{50} values of the β_1 adrenoceptor and the β_2 adrenoceptor), or
- iii) by a combination of both.
- iv) Furthermore, these characteristics should be combined with a potent functional activity on the β_2 adrenoceptor (e.g. EC_{50} in the low nM range [usually < 10 nM]) and a sufficient intrinsic activity (in the range of 50-100%).

Additionally to the general criteria i-iv, only compounds showing sufficient efficacy in preclinical animal models mirroring the bronchoconstriction seen in COPD patients, can be regarded for use in a clinical setting.

IN VITRO EFFICACY AND POTENCY

The examples from the instant application were tested in cellular functional assays to determine their potency, efficacy, and selectivity for the human β_2 adrenoceptor. In the assays, the rise of intracellular cAMP induced by a β -agonist is measured in CHO cell lines expressing either the recombinant human β_1 or β_2 adrenoceptor, respectively, and the EC_{50} values and the intrinsic activities (as a marker for efficacy) for the two β adrenergic receptors are determined. An exact description is given in the attached Standard Operating Procedure in appendix 1.

The data obtained in these assays for the examples 9, 10, 11 and 12 from the instant application are summarized in table 1 and are discussed in the following.

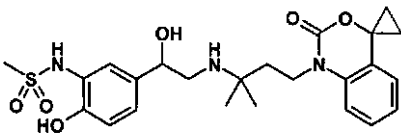
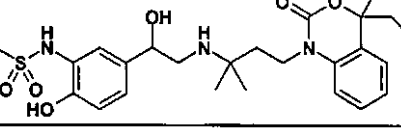
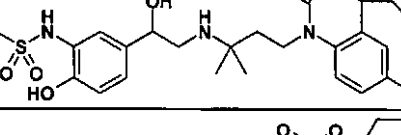
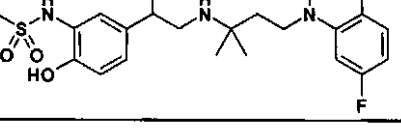
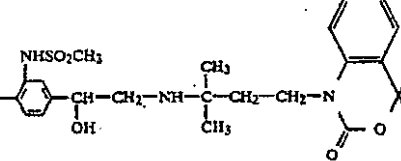
- Example 9 is a partial agonist of the β_2 receptor with an intrinsic activity compared to isoprenaline of 84 % and an EC_{50} value of 2.6 nM. Furthermore, the compound displays a 38-fold higher potency on β_2 receptor than on the β_1 receptor.
- Example 10 of the instant application is a potent, partial agonist of the human β_2 receptor with an EC_{50} value of 8.6 nM and displays an excellent selectivity over the β_1 receptor. The calculated selectivity ($EC_{50}(\beta_1) / EC_{50}(\beta_2)$) is 106.

- Also example 11 of the instant application is a potent, partial agonist of the human β_2 receptor with an EC_{50} value of 5.7 nM and an excellent selectivity over the β_1 receptor ($EC_{50}(\beta_1) / EC_{50}(\beta_2)$) of 119.
- Example 12 shows similar *in vitro* characteristics. Again, this compound is a partial agonist of the β_2 adrenoceptor with an EC_{50} value of 8.7 nM. The calculated selectivity ($EC_{50}(\beta_1) / EC_{50}(\beta_2)$) is 16. The modest selectivity is compensated by a very low, nearly negligible intrinsic activity on the β_1 receptor of 11 %.

Example A is a partial agonist of the β_2 adrenoceptor with an intrinsic activity of 80% compared to isoprenaline and an EC_{50} value of 2.7 nM. The calculated selectivity ($EC_{50}(\beta_1) / EC_{50}(\beta_2)$) is 66. Thus the compound exerts the *in vitro* properties desired for a betamimetic in these assays.

Therefore, all examples have excellent *in vitro* properties and the desired profile for a betamimetic in these assays.

Table 1: Results from the cellular assay measuring the cAMP accumulation in CHO cell lines expressing cloned human β_1 or β_2 adrenergic receptors. Examples from the instant application were tested in this cell assay and the results are summarized. IA = intrinsic activity in comparison to isoprenaline (IA isoprenaline = 100%). For more details see the Standard Operating Procedure in appendix 1.

Example #	Structure	β_1 IA [%]	β_1 EC_{50} [nM]	β_2 IA [%]	β_2 EC_{50} [nM]
9		31	100	84	2.6
10		49	909	58	8.6
11		8	716	51	5.7
12		11	139	53	8.7
A	 Example 11 column 11 of US 4,341,778	35	179	80	2.7

IN VIVO EFFICACY IN GUINEA PIGS AND DOGS

A) Guinea pig model

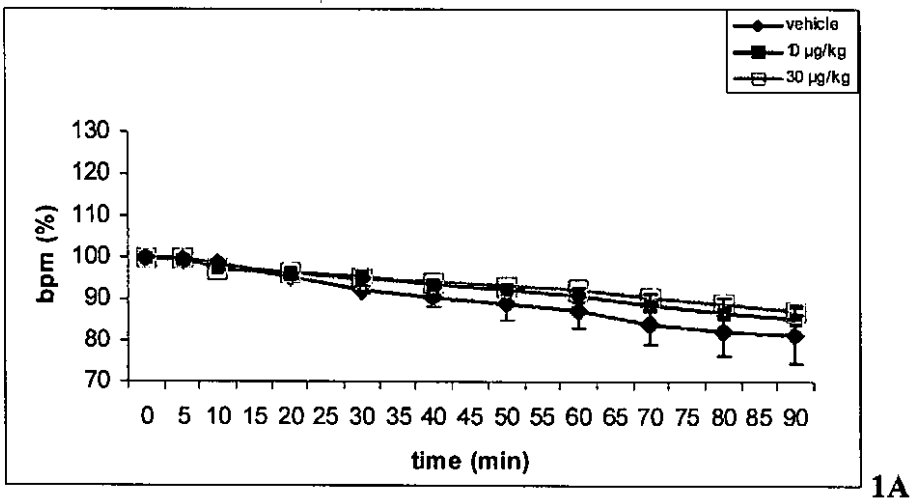
Examples from the instant application were also tested in a bronchoconstriction model in guinea pigs (Konzett-Roessler model). In this model, compounds were applied intra-tracheally to anaesthetized guinea pigs and bronchoconstriction was induced by intravenous application of acetylcholine at various time-points after administration of the compound.

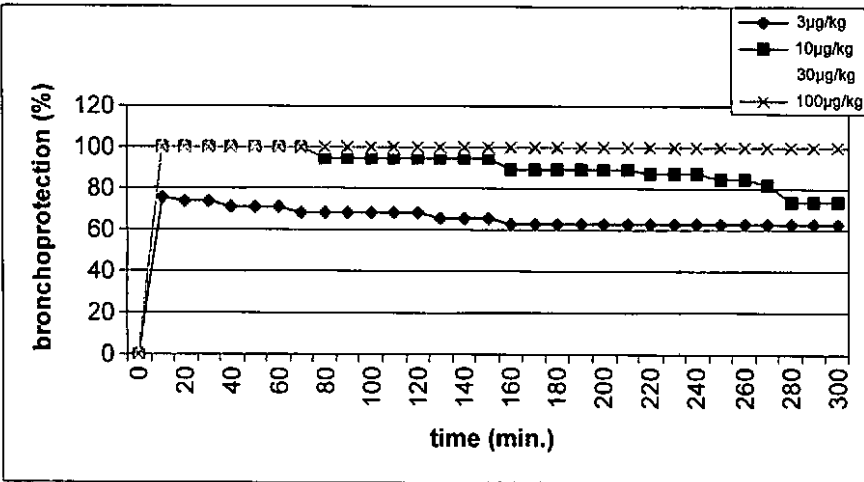
This model is widely used to test the *in vivo* efficacy, potency, duration of action and systemic side effect profile of bronchodilators, such as beta-agonists. The model is a good predictor for the efficacy of compounds in human airway diseases such as chronic obstructive pulmonary disease since the vagal cholinergic tone is an important contributor for the reduced airflow in COPD patients. An exact description of the model is given in appendix 1. The results of these tests are listed in table 2. Compounds suitable for use in COPD should show in this model

- i) a strong, ideally complete (= 100%) bronchoprotection at doses $\leq 30 \mu\text{g/kg}$,
- ii) a duration of action of more than 5 h (= should show bronchoprotection at this time-point),
- iii) a good therapeutic window (= should be devoid of systemic side effects, e.g. an increase in heart rate).

In this model, the fully effective dose (FED) corresponds to the first dose producing a full inhibition of the acetylcholine-induced bronchospasm (= 100 % bronchoprotection). The therapeutic window corresponds to the ratio between the first dose producing an increase in heart rate (= side-effect dose: SED) and the FED. An increase in heart rate above 10% of the heart rate recorded before the inhalation of the drug is regarded as pharmacologically significant.

Figure 1: Pharmacological profile of example 10 from the instant application in the Konzett-Roessler model in guinea pigs. Example 10 from the instant application was tested in this model at different doses and the bronchoprotection (1B) and heart rate in beats per minute (bpm; 1A) were determined as described in the Standard Operating Procedure in appendix 1.

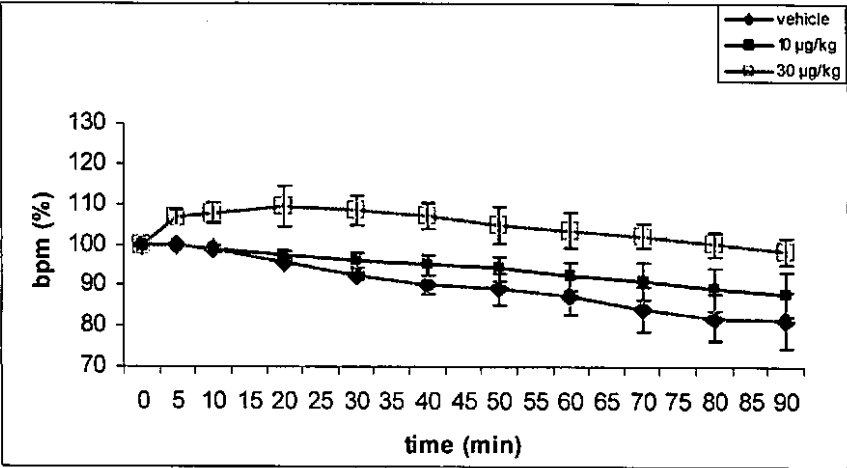




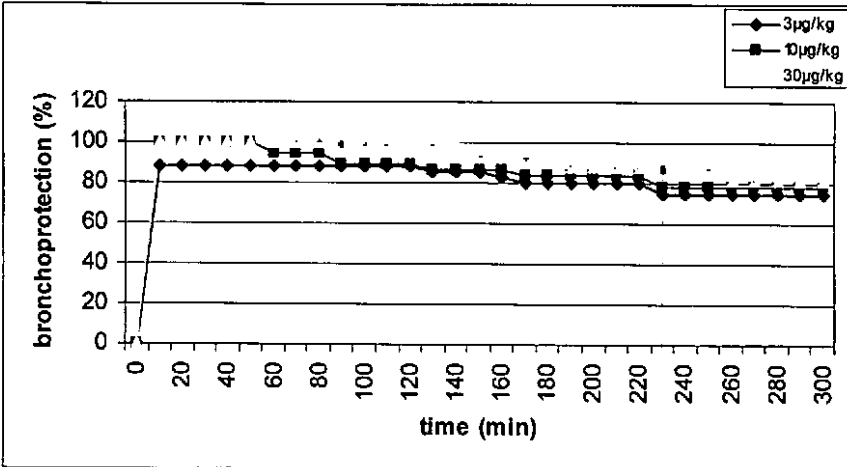
1B

As shown in figure 1, **example 10** of the instant application shows in this model a dose dependent bronchoconstriction over a period of 5 h. The FED was determined as 10 µg/kg. As shown in figure 1A, no increase in heart rate was observed either at the FED (10 µg/kg) or a three-fold higher dose. An increase in heart rate was observed at a dose of 100 µg/kg (data not shown, see table 3). Therefore, the therapeutic window (SED/FED) is defined as 10.

Figure X: Pharmacological profile of **example A** from XXX in the Konzett-Roessler model in guinea pigs. **Example A** was tested in this model at different doses and the bronchoprotection (A) and heart rate in beats per minute (bpm; B) were determined as described in the Standard Operating Procedure in appendix 1.



XA



XB

As shown in figure X, **example A** shows in this model a dose dependent bronchoconstriction over a period of 5 h (figure X B). The FED was determined as 10 µg/kg. As shown in figure XA, suprisingly a relevant increase in heart rate was observed at the dose of 30 µg/kg. Therefore, the therapeutic window (SED/FED) is defined as 3. Based in this results the preclinical pharmacological profile of compound A is surprisingly inferior to compound 10 of the current application.

The pharmacological profiles of example 10, 11 and A from the instant application are summarized in table 3.

Table 3: Summary of results from the profiling of example 10 and 11 of the current application and of compound A in the Konzett-Roessler model. The two examples from the instant application were tested in this model at various doses. The heart rate (HR) recorded 20 min after inhalation of the drug is given as the percentage of the HR (beats/ min) recorderd before drug inhalation. The fully effective dose (FED) corresponds to the first dose producing a full inhibition of the acetylcholine-induced bronchospasm. The therapeutic window corresponds to the ratio between the first dosc producing an increase in heart rate (= side-effect dose: SED) and the FED. For details see the Standard Operating Procedure below (HR: heart rate, FED: fully effective dose, SE: first dose showing increase in HR > 10 %, SE/FED: side-effect / fully effective dose, ND: not determined)

#	HR increase [%] at 1 µg/kg	HR increase [%] at 3 µg/kg	HR increase [%] at 10 µg/kg	HR increase [%] at 30 µg/kg	HR increase [%] at 100 µg/kg	FED µg/kg	SED/ FED
10	ND	1 %	1 %	0 %	11 %	10	10
11	ND	1 %	0 %	5 %	10 %	30	= 3
A	ND	0 %	1 %	14 %	ND	10	= 3

B) Dog model

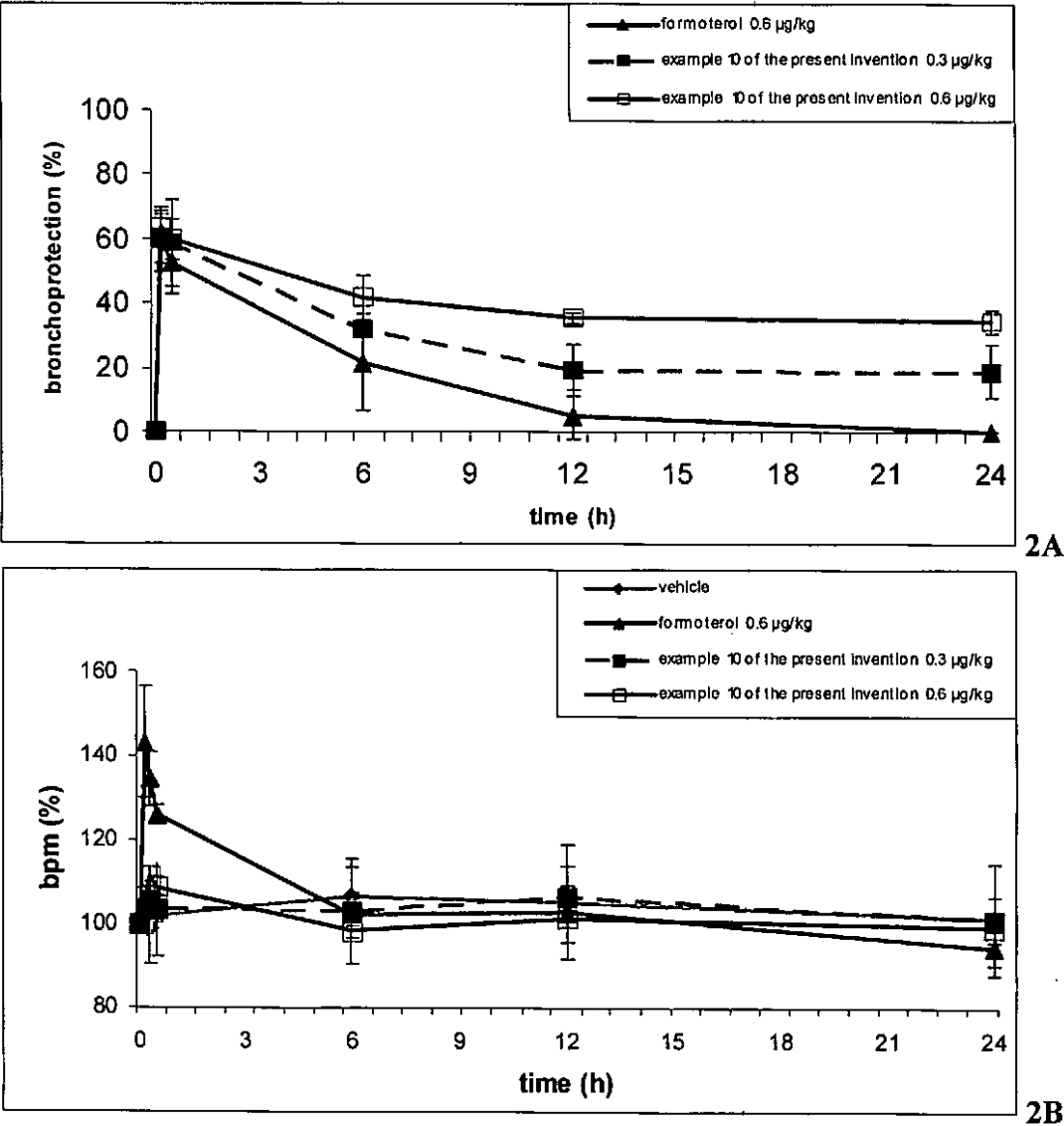
Furthermore, the description of the patent application discloses the claimed compounds as suitable for a **once-a-day** treatment in COPD. To address this topic, **example 10** of the instant application has been tested in a bronchoconstriction model in dogs. In this model test, compounds are administered by inhalation using the Respimat® inhaler directly connected to the tracheal tube of anaesthetized ventilated dogs and bronchoconstriction is induced by intravenous injections of acetylcholine at different time points before and after compound administration. Furthermore, the clinically dose-limiting systemic pharmacodynamic effects of beta agonists, namely increase in heart rate and hypokalemia are determined. This model is predictive for the clinical situation since:

- i) it allows to test for the primary pharmacodynamic effects of beta agonists (= bronchodilation; referred to as bronchoprotection in the figure below),
- ii) an increased cholinergic tone is involved in the pathophysiology of COPD
- iii) it allows to determine the clinically dose-limiting systemic pharmacodynamic effects of beta agonists e.g. increase in heart rate and hypokalemia (for details please refer to appendix 1).

The duration of action of **example 10** was compared in this preclinical model to the duration of action of formoterol, a beta agonist with a twice a day treatment regime, which served as a

gold standard at the filing date of the present application. Both compounds were tested at their maximal effective dose.

Figure 2: Bronchoprotection and cardiovascular effects of **example 10** and **formoterol** in dogs over 24 h. A) Duration of action of **example 10** and **formoterol** in anesthetized dogs. **Example 10** and **formoterol** were administered intra-tracheally using the Respimat® Inhaler at a dose of 0.3 and 0.6 µg/kg (**example 10**) or 0.6 µg/kg (**formoterol**). Bronchoconstriction was induced 0.1, 0.5, 1, 6, 12 and 24 h after administration. Bronchoprotection is expressed as a percentage of inhibition of the increase in pulmonary resistance induced by acetylcholine preceding drug inhalation. B) Cardiovascular effects of **example 10** and **formoterol** in anaesthetized dogs over 24 h. Heart rate profiles of **example 10** (0.3 µg/kg and 0.6 µg/kg) and **formoterol** (0.6 µg/kg) 0.1, 0.5, 1, 6, 12 and 24 h after inhaled administration using the Respimat® Inhaler are shown. The increase in heart rate is expressed as a percentage of heart rate (beats/min) recorded before drug inhalation. Each value represents the mean ± SD (n = 2-4).



The results show that compound **10** exerts a duration of action over 24 h (see figure 2A). In contrast, **formoterol** loses activity already after 12 h. These data are in agreement with the twice a day dosing regimen for **formoterol** seen in the clinical setting and show that **example 10** is suitable for once-daily dosing. Furthermore, the data show that **example 10** exerts a systemic pharmacodynamic effect profile superior to **formoterol**. This is demonstrated by the significant increase in heart rate observed for **formoterol** but not **example 10** (see figure 2B).

Taken together, the data confirm that the pharmaceutical composition including **example 10** of the current claim set fulfils the characteristics of a beta agonist with a once-a-day treatment regimen.

APPENDIX 1: EXPERIMENTAL PROTOCOLS

CHO β 1

Determination of Beta 1 adrenoreceptor agonism, by stimulation of cAMP synthesis in CHO cells. Determination of EC 50 and intrinsic activity (IA)

Materials: PBS (Cat.No.#14190-169), DMEM medium (CatNo.#31966-021), Pen/Strep (CatNo.#15140-106), MEM (Cat.No.#11140-035), Geneticin (Cat.No.#10131-027), Trypsin/EDTA (Cat.No.#25300-054) and Versene (Cat.No.#15040-033) were from Invitrogen. FBS was from Bio Whittaker (CatNo.#14-602F). HBSS 10x (Cat.No.#14065-049) was purchased from Gibco BRL. Tween 20 (Cat.No.#37240) was ordered from Serva. BSA (Cat.No.# A-7030), DMSO (Cat.No.# D-5879), NaCl (Cat.No.# S-3014), IBMX (Cat.No.# I-7018), Isoprenaline (Cat.No.# I-5627) and cAMP (Cat.No.# A-6885) were from Sigma. The 96-half area flat bottom plates were purchased from Corning (# 3697). The OptiPlate 384 well plate was from Packard Bioscience (#6007290). The AlphaScreen cAMP Detection Kit (Cat.-No. 6760600M) was from BioSignal Packard. All other materials were of highest grade commercially available.

The CHO cells expressing the human beta 1 adrenergic receptor were generated by standard molecular biology methods. These cells express the human beta1 adrenergic receptor. Sequence described in (Friele et al., Proc. Natl. Acad. Sci. USA 84, 7920-7924, 1987).

Method:

Culture medium:	DMEM, 10% FBS, 1% Pen/Strep, 1% MEM, 1mg/ml Geneticin
Stimulation buffer:	0.1%BSA, 0.5 mM IBMX, 5 mM Hepes, 1x HBSS (10x)
Lysis buffer:	0.3% Tween 20, 5 mM Hepes, 0.1% BSA
Bead buffer:	0.1%BSA, 6% 50mM Hepes, 4% HBSS

CHO cells are grown to 70-80 % of confluence. After washing the cells 3x with PBS, cells were detached by using Versene and resuspended in 10 ml of stimulation buffer. After centrifugation (5 min 900 rpm), the supernatant was removed and the cells were resuspended in 10 ml of stimulation buffer followed by another centrifugation step. Then cells were adjusted to 0.65×10^6 cells/ml using stimulation buffer using the Casy system.

10 μ l of the test compounds diluted in 0.9%NaCl (final concentration of compounds: 0.1 nM to 10 μ M; DMSO 1%) were added to the 96 well plate and were mixed with 20 μ l of the cell suspension. After incubation of 30 minutes at RT, 20 μ l of lysis buffer are added followed by an incubation of at least 30 min on ice. 5 μ l of the lysed cell suspension are then transferred in quadruplicate into a 384 well plate, followed by the addition of 10 μ l acceptor beads (final concentration 15 μ g/ml in bead buffer). Plates are incubated at RT for 30 min in the dark before 10 μ l of the donor beads (final concentration 20 μ g/ml in bead buffer) including

biotinylated cAMP (final concentration 10 nM) are added. After incubation of at least 2 h, the assay is measured using a Fusion reader (Packard Bioscience).

The resulting Alpha Screen Counts (=ASC) are directly recalculated as cAMP production using a cAMP standard curve.

Each assay microtiter plate contains wells with vehicle controls (1% DMSO, unstimulated cells) as reference for non stimulation (0% CTL; low values) and wells stimulated with isoprenaline (1 x 10⁻⁵ M final concentration) as positive controls (100% CTL; high values).

Determination of the intrinsic activity (IA): The analysis of the data is performed by the calculation of the percentage of cAMP in the presence of the test compound compared to cAMP generated of the positive control:

$$((\text{cAMP}(\text{sample}) - \text{cAMP}(\text{low value})) * 100 / (\text{cAMP}(\text{high value}) - \text{cAMP}(\text{low value})))$$

An agonist of the beta 1 receptor will give IA values between 0 % CTL (no stimulation) and usually < 200 % CTL (100% corresponding to reference stimulation by 1 x 10⁻⁵ M final concentration of isoprenaline)

Determination of the effective concentration 50 (EC₅₀): Is determined by applying the % CTL data obtained at the various concentrations of each compound to graph pad prism software. Data are reported as nanomolar (nM).

CHO β2

Determination of Beta 2 adrenoreceptor agonism, by stimulation of cAMP synthesis in CHO cells. EC50 and IA determination

Materials: PBS (Cat.No.#14190-169), DMEM medium (CatNo.#31966-021), Pen/Strep (CatNo.#15140-106), MEM (Cat.No.#11140-035), Geneticin (Cat.No.#10131-027), Trypsin/EDTA (Cat.No.#25300-054) and Versene (Cat.No.#15040-033) were from Invitrogen. FBS was from Bio Whittaker (CatNo.#14-602F). HBSS 10x (Cat.No.#14065-049) was purchased from Gibco BRL. Tween 20 (Cat.No.#37240) was ordered from Serva. BSA (Cat.No.# A-7030), DMSO (Cat.No.# D-5879), NaCl (Cat.No.# S-3014), IBMX (Cat.No.# I-7018), Isoprenaline (Cat.No.# I-5627) and cAMP (Cat.No.# A-6885) were from Sigma. The 96-half area flat bottom plates were purchased from Corning (# 3697). The OptiPlate 384 well plate was from Packard Bioscience (#6007290). The AlphaScreen cAMP Detection Kit (Cat.-No. 6760600M) was from BioSignal Packard. All other materials were of highest grade commercially available.

The CHO cells expressing the human beta 2 adrenergic receptor were generated by standard molecular biology techniques.

Method:

Culture medium:	DMEM, 10% FBS, 1% Pen/Strep, 1% MEM, 1mg/ml Geneticin
Stimulation buffer:	0.1%BSA, 0.5 mM IBMX, 5 mM Hepes, 1x HBSS (10x)
Lysis buffer:	0.3% Tween 20, 5 mM Hepes, 0.1% BSA
Bead buffer:	0.1%BSA, 6% 50mM Hepes, 4% HBSS

CHO cells are grown to 70-80 % of confluence. After washing the cells 3x with PBS, cells were detached by using Versene and resuspended in 10 ml of stimulation buffer. After centrifugation (5 min 900 rpm), the supernatant was removed and the cells were resuspended

in 10 ml of stimulation buffer followed by another centrifugation step. Then cells were adjusted to 0.65×10^6 cells/ml using stimulation buffer using the Casy system.

10 μ l of the test compounds diluted in 0.9%NaCl (final concentration of compounds: 0.1 nM to 10 μ M; DMSO 1%) were added to the 96 well plate and were mixed with 20 μ l of the cell suspension. After incubation of 30 minutes at RT, 20 μ l of lysis buffer are added followed by an incubation of at least 30 min on ice. 5 μ l of the lysed cell suspension are then transferred in quadruplicate into a 384 well plate, followed by the addition of 10 μ l acceptor beads (final concentration 15 μ g/ml in bead buffer). Plates are incubated at RT for 30 min in the dark before 10 μ l of the donor beads (final concentration 20 μ g/ml in bead buffer) including biotinylated cAMP (final concentration 10 nM) are added. After incubation of at least 2 h, the assay is measured using a Fusion reader (Packard Bioscience).

The resulting Alpha Screen Counts (=ASC) are directly recalculated as cAMP production using a cAMP standard curve.

Each assay microtiter plate contains wells with vehicle controls (1% DMSO, unstimulated cells) as reference for non stimulation (0% CTL; low values) and wells stimulated with isoprenaline (1×10^{-5} M final concentration) as positive controls (100% CTL; high values).

Determination of the intrinsic activity (IA): The analysis of the data is performed by the calculation of the percentage of cAMP in the presence of the test compound compared to cAMP generated of the positive control:

$$((\text{cAMP}(\text{sample}) - \text{cAMP}(\text{low value})) * 100 / (\text{cAMP}(\text{high value}) - \text{cAMP}(\text{low value})))$$

An agonist of the beta 1 receptor will give IA values between 0 % CTL (no stimulation) and usually < 200 % CTL (100% corresponding to reference stimulation by 1×10^{-5} M final concentration of isoprenaline)

Determination of the effective concentration 50 (EC₅₀): Is determined by applying the % CTL data obtained at the various concentrations of each compound to graph pad prizm software. Data are reported as nanomolar (nM).

BRONCHOCONSTRICTION MODEL IN GUINEA PIGS ACCORDING TO KONZETT-ROESSELER

Animals

Male and female Dunkin-Harley guinea pigs are obtained from the Experimental Animal Breeding Centre of Harlan Winkelmann (Germany). After fasting overnight, but with free access to drinking water, animals with body weight of 400-500g are used.

Anesthesia and preparation

Anesthesia is induced by intraperitoneal injection of 50 mg/kg pentobarbital. Anesthesia is prolonged by intravenous infusion of pentobarbital (15 mg/kg/h) via the jugular vein. A tracheal cannula is introduced after tracheotomy for artificial ventilation. The internal jugular vein is cannulated for acetylcholine injection.

Instrumentation

Bronchospasm is recorded with a modified version of the method of Konzett-Rößler described by Walland et al, 1997. The animals are ventilated by means of a piston pump (starling ventilator, Hugo Sachs Elektronik, Germany) at a stroke volume of 1ml/100g body weight and at a rate of 60 strokes per min. The tubing which connected the tracheal cannula with the ventilator is provided with a branch leading to the bronchospasm transducer (bronchospasm transducer 7020, Ugo Basile, Italy) The air flow measurement is based on the hot wire anemometer principle. Any air flow will reduce the temperature of the wire, thereby decreasing its resistance in proportion of the flow. As the wire element is one arm of a Wheatstone bridge, variation in resistance generate a proportional voltage output which feeds the amplifier/recording system (Notocord-hem, Notocord, France).

Blood pressure and heart rate are monitored from a carotid artery in order to check the anesthesia and the variability of the preparation.

Experimental protocol

30 minutes after the beginning of air flow and blood pressure measurements, acetylcholine is injected every 10 minutes in order to get transient and reliable bronchospasm. Bronchospasms are recorded as overflow (in ml). Intensity of overflow recorded, has to be included between 1 and 2 ml in order to have a standard response for all animals. That means, at the beginning of the study, we have to find the appropriate dose of acetylcholine (usually, between 8 and 12 µg/kg i.v.) in order to get the right dose which induce an overflow of about 1 to 2ml. After three stable acetylcholine bronchospasms, the long acting β_2 -agonist is administrated via the tracheal cannula using a soft mist inhaler. This administration is followed by a recording period for 5 hours where acetylcholine is injected every 10 minutes. 4 groups of animals are used including a negative control group (vehicle), and three doses of the test compound (range of doses: 0.1 to 30 µg/kg inhaled). Each group comprises 2 guinea-pigs. At the end of the experiment, animals are euthanized by an overdose of pentobarbital (100 mg/kg i.v.). Intravenous injection of acetylcholine in basal conditions induces a bronchoconstriction characterized by an increase in overflow. Bronchoprotection of the drug (i.e. inhibition of acetylcholine bronchoconstrictor response) is expressed as a percentage of inhibition of the increase in overflow induced by acetylcholine preceding drug inhalation. After drug administration, acetylcholine is injected intravenously every 10 minutes for 5 h.

Blood pressure and heart rate are monitored from a carotid artery in order to monitor the anaesthesia and the systemic pharmacological activity of the test compounds. Heart rate is expressed as a percentage of the pre-value (beats per minutes) preceding the drug inhalation. The increase in heart rate expressed in percent corresponds to the difference of heart rate between the vehicle group and the treated group recorded 20 minutes after drug inhalation. An increase in heart rate of at least 10 % or more in the treated group compared to the vehicle group, is considered to be relevant as a systemic pharmacological activity (tachycardia).

Compounds

The compound are dissolved in physiological saline or in a mixture of distilled water and ethanol (40/60, v/v) at concentrations permitting administrations of 0.1 – 30 µg/kg per actuation (10 µl) of the soft mist inhaler. According to the stability of the compound in solution, a stock solution of 3mg/10ml or 10mg/10ml can be prepared and reused the whole week.

Acetylcholine (Acetylcholine ophthalmicum Dispers) is from Dispersa GmbH (Germering, Germany). Pentobarbitone (Narcoren®) is from Rhone Merieux GmbH, (88471 Laupheim,

Germany). Formoterol fumarate obtained from Boehringer Ingelheim Pharma KG is dissolved in a mixture of distilled water and ethanol (40/60, v/v).

Data reporting

Bronchoprotection is expressed as a percentage of inhibition of the increase in overflow induced by acetylcholine before drug inhalation. The fully effective dose (FED), which can be used as a marker for the potency of a test compound is reported as the lowest dose (in $\mu\text{g/kg}$) resulting in a complete bronchoprotection.

DETERMINATION OF BRONCHOCONSTRICTION AND SYSTEMIC PHARMACODYNAMIC EFFECTS IN DOGS OVER 24 HOURS

Animals

Male and female Beagle dogs with a body weight ranging from 10 – 14 kg are obtained from the in-house breeding station. The animals are kept in the kennel of the local animal house, fasted overnight before the experiment, but have free access to drinking water.

Anaesthesia and Preparation

Dogs are anaesthetized repeatedly for measuring lung function 0.1 h, 0.5 h, 6h, 12 h and 24 hours after administration of the test compound. In the meantime the dogs are allowed to wake up.

Anaesthesia is induced by intravenous bolus injection of 10 mg/kg propofol followed by an intravenous infusion of 30 mg/kg/h propofol into the cephalic vein. The dogs are intubated and then ventilated with volume-controlled pressure with a mixture of room air and oxygen (3:1) using a Siemens respirator at a rate of 15 strokes per minute. Optimal ventilation is assured by regular measurement of acid-base status and oxygen saturation in the blood. The contralateral cephalic vein is prepared for acetylcholine (ACh) injections. Adhesive electrodes are placed onto the locally shaved skin of the four legs and are used for ECG recording the limb leads for evaluation of heart rate.

Transpulmonary pressure is monitored by means of a differential pressure transducer (Sensortech GmbH, Puchheim, Germany) using a catheter connected with the endotracheal tube. A Fleisch-tube type 1 (Hugo Sachs Elektronik- Harvard Apparatus GmbH, March Hugstetten, Germany) is connected to the endotracheal tube for pneumotachographic measurement of respiratory flow.

Body temperature is maintained at 37-38°C by means of a heating bed.

Blood Assays

Blood gas and oxygen status as well as the concentrations of sodium, potassium, calcium, chloride, glucose and lactate in heparinized arterial blood samples are assayed with an ABL 605 analyzer (Radiometer Copenhagen, Denmark).

Experimental Protocol

A control period of 30 min follows the completion of the instrumentation. Acetylcholine is repeatedly injected at a dose of 10 $\mu\text{g/kg}$ for the induction of transient bronchospasm (approx. 30 % increase in bronchial resistance) at times: -0.75 h, -0.5 h, -0.25 h, 0.1 h, 0.5 h, 6 h, 12 h and 24 h after the initial treatment. Cardiovascular parameters are evaluated at the same times

immediately before induction of bronchospasm. At the end of the experiment, dogs wake up and will be reused after a washout period of at least 4 weeks.

Drugs

The compound is dissolved in water for injection. According to the stability of the compound in solution, a stock solution can be prepared and reused the whole week.

Acetylcholine (Acetylcholine ophthalmicum Dispers) is from Dispersa GmbH (Germering, Germany). Propofol (Propofol-Lipuro 2 %) is from B Braun Melsungen AG, (D-34209 Melsungen, Germany). Propranololhydrochlorid (Obsidan) is from Alpharma-Isis GmbH & Co. KG (D-40764 Langenfeld, Germany).