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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-109913- -00005-0000

Fecha: 2011-06-28 15:55:17 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 523 RESPUESTA Folios: 75

Señor Director
DIRECCIÓN DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite : 011
Evento : 378
Actuación : 444

Referencia : Expediente No. 07109913
Asunto : Solicitud de patente para: **DIHIDROTIENOPYRIMIDINAS
PARA EL TRATAMIENTO DE ENFERMEDADES
INFLAMATORIAS**
Solicitante : BOEHRINGER INGELHEIM INTERNATIONAL GMBH
Clase : A61K 031/519, A61P 011/000, C07D 495/004
Fecha solicitud : 18 de octubre de 2007

1. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, de conformidad con la sustitución de poder otorgada a mí por el doctor Emilio FERRERO, me refiero al oficio No. **5891** notificado por fijación en lista del 01 de abril de 2011.

2. Mediante oficio No. **5891** 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 Reivindicaciones

Menciona el Examinador que la reivindicación 1 no es concisa por cuanto incluye términos como "enantiómeros", "sal", "hidratos" o "solvatos" y los cuales no son especificados en el pliego reivindicatorio. Se sugiere definir con exactitud los compuestos a los cuáles se refieren.

De otro lado, se ha objetado la materia de las reivindicaciones 24 a 27 por cuanto se relaciona con usos.

2.2 Nivel inventivo

Considera el Examinador que el documento D1: US2003016275 afecta el nivel inventivo de la presente solicitud.

De acuerdo con los argumentos expuestos, la persona medianamente versada en la materia llegaría de manera obvia a la presente invención modificando el compuesto conocido en D1 para obtener compuestos con actividad sobre la enzima PDE-4.

3. Respuesta a las objeciones

Con el objeto de atender las objeciones arriba mencionadas, se desea poner de presente las siguientes modificaciones, aclaraciones y argumentos:

3.1 Modificación de una solicitud en trámite

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 pliego reivindicatorio conformado por 23 reivindicaciones.

Así, siguiendo las amables sugerencias del Examinador, se ha eliminado de las reivindicaciones 1 a 18 la frase "así como sus sales, diastereómeros, enantiómeros, racematos, hidratos o solvatos farmacológicamente tolerables" y en su lugar, se ha incluido el siguiente párrafo:

"así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico".

Esta modificación está soportada en la descripción, página 45, líneas 14 a 22.

De otro lado y con el ánimo de definir mejor los intermediarios de las reivindicaciones 19 y 20, se ha procedido a incluir las fórmulas de éstos en dichas reivindicaciones.

Finalmente, se han eliminado las reivindicaciones relacionadas con usos.

Estas modificaciones permiten entonces presentar un pliego más claro y conciso y de conformidad con las disposiciones de la Decisión 486. Adicionalmente, este pliego reivindicatorio permite superar las objeciones comunicadas por el Examinador.

3.2 Argumentos a favor del nivel inventivo de la solicitud

De acuerdo con el artículo 18 de la Decisión 486:

Artículo 18.- Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.

Siguiendo las directrices de la Decisión 486 y, específicamente del artículo 18, se considera que una invención es inventiva cuando no resulta evidente a la vista de las enseñanzas de la técnica anterior, para una persona medianamente versada en la materia.

De acuerdo con el Tribunal de Justicia de la Comunidad Andina: "Lo obvio es aquello que salta a la vista de manera clara y directa; lo que se encuentra o pone delante de los ojos, muy claro o que no tiene dificultad. Lo evidente consiste en la certeza clara y manifiesta de la que no se puede dudar; cierto, claro, patente y sin la menor duda (véase proceso 13-IP-2010).

Por consiguiente, para que una anterioridad pueda afectar el nivel inventivo de una solicitud de patente, debe comprender enseñanzas claras y directas (obvias) para que la persona medianamente versada en la materia pueda considerarlas y aplicarlas para un fin determinado.

Lo anterior es corroborado por el Tribunal de Justicia de la Comunidad Andina cuando establece: "En este orden de ideas, se puede concluir que una invención goza de nivel inventivo cuando a los ojos de un experto medio en el asunto de que se trate, se necesita algo más que la simple aplicación de los conocimientos técnicos en la materia para llegar a ella, es decir, que de conformidad con el estado de la técnica el invento no es consecuencia clara y directa de dicho estado" (véase proceso 13-IP-2010) .

Para el presente caso, el Examinador ha citado el documento D1 afirmando que afecta el nivel inventivo de la presente solicitud.

Sin embargo, respetuosamente nos apartamos de la opinión del Examinador, por lo que consideramos que la presente solicitud no resulta en ningún caso obvia a la luz del

documento D1 y que además, los compuestos reivindicados ofrecen un efecto técnico superior inesperado sobre el arte previo, tal como se detalla a continuación:

En primer lugar deseamos llamar la atención del Examinador sobre el hecho de que el documento D1 fue incorrectamente citado, por lo que no corresponde al No. de solicitud US2003016275 sino al No. US2003016295. Los argumentos aquí presentados hacen entonces referencia a dicho último documento.

Ahora bien, los compuestos de la presente solicitud y los compuestos del documento D1 difieren en la ausencia de un sustituyente piperazino en la cuarta posición (entre los dos átomos de nitrógeno de la tienopirimidina) y la ausencia del doble enlace en la posición-6,7 de la dihidrotienopirimidina. Por el contrario, los compuestos de D1 son tienopirimidinas con un enlace doble en la posición-6,7.

De otro lado, se presenta junto con este memorial una prueba comparativa que demuestra que la ausencia del enlace doble en la posición-6,7 de las dihidrotienopirimidinas de la presente solicitud resultan en valores IC50 menores en un factor entre 5 y 20, comparados con los compuestos específicamente reivindicados. Consecuentemente, las 6,7-dihidrotienopirimidinas (que no comprenden el enlace doble en la posición-6,7) tal como se reivindican en la presente solicitud muestran un valor IC50 menor comparado con el valor obtenido para las tienopirimidinas que comprenden dicho enlace doble en la posición-6,7. Consecuentemente, los compuestos de conformidad con la presente invención muestran un potencial mayor para inhibir la enzima PDE-4 (ver comparaciones de dihidrotienopirimidinas de la presente solicitud y sus respectivas tienopirimidinas que incluyen la determinación de los valores IC50 respectivos).

Adicionalmente, se adjuntan cinco documentos del estado de la técnica que demuestran que los inhibidores de PDE-4 efectivos son útiles para la terapia de enfermedades respiratorias (tales como COPD y asma), cáncer (por ejemplo de colon), enfermedades gastrointestinales (tal como la enfermedad de Crohn y colitis ulcerativa) y enfermedades neuro-degenerativas (tal como esclerosis múltiple, Alzheimer y de cognición).

Tres de los documentos del arte previo muestran claramente que las 6,7-tienopirimidinas de la presente invención (las cuales resultan más efectivas como inhibidores de PDE-4 comparado con las tienopirimidinas del documento D1), son particularmente apropiadas para tratar todas las enfermedades mencionadas en la presente solicitud.

Consecuentemente, dado que el documento D1 no sugiere las diferencias técnicas que caracterizan los compuestos de la presente solicitud y mucho menos sugiere que dichas diferencias técnicas generan un efecto técnico superior sobre los documentos del arte previo, se considera que la presente invención resulta inventiva sobre el documento D1.

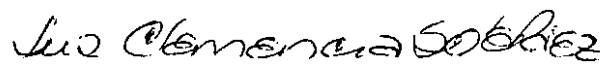
Por lo tanto, la objeción formulada a este respecto se ve superada.

5. Puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

6. ANEXOS

- Nuevo pliego reivindicatorio conformado por 23 reivindicaciones
- Formulario de modificaciones en solicitud pendiente
- Recibo por concepto de presentación de 13 reivindicaciones adicionales a la décima por valor de \$351.000
- 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
- Anexo 1: Prueba comparativa
- Anexo 2: Banner K. et al. *PDE4 INHIBITION: A NOVEL APPROACH FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE*
- Anexo 3: Huang Z. et al. *THE NEXT GENERATION OF PDE4 INHIBITORS*
- Anexo 4: Murata K. et al. *CYCLIC AMP SPECIFIC PHOSPHODIESTERASE ACTIVITY AND COLON CANCER CELL MOTILITY*
- Anexo 5: Spina D. *THE POTENTIAL OF PDE4 INHIBITORS IN ASTHMA OR COPD*
- Anexo 6: Vignola A. *PDE4 INHIBITORS IN COPD-A MORE SELECTIVE APPROACH TO TREATMENT*

Señor Director,


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

**Industria y Comercio**

SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

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: Binger Strasse 173,
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 PÁEZ**Dirección:** Carrera 4ª. No. 72-35**Teléfono:** 347 36 11**Fax:** 211 86 50**E-mail:** clientes@cavelier.com**Domicilio:** Bogotá, Colombia.**IDENTIFICACIÓN**C.C. ☒ NIT ☐
C.E. ☐ Otro ☐

Cual _____

Número 35.456.344 T. P. 23.555**4****Número de expediente:** 07.109.913**5****Descripción de la corrección o modificación solicitada:**

Presento nuevo capítulo reivindicatorio compuesto por 23 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.** 11-61493 **Fecha:** 24 de junio de 2011**Comprobante de pago No.** 11-61494 **Fecha:** 24 de junio de 2011**Comprobante de pago No.** 11-61495 **Fecha:** 24 de junio de 2011

7

Anexos



Comprobante de pago de la tasa por \$118.000, por concepto de modificaciones.



Comprobante de pago por 13 reivindicaciones adicionales después de la décima reivindicación por \$351.000.00.



Poderes, si fuere el caso



Copia auténtica de la sustitución que del poder otorgado por la Sociedad Solicitante hizo el Doctor EMILIO FERRERO a mi favor, para lo cual solicito me sea reconocida personería y así continuar con el trámite de la presente solicitud en favor del Solicitante.

8

NOMBRE: LUZ CLEMENCIA DE PÁEZ

FIRMA:

Luz Clemencia de PáezC.C. 35.456.344 de Usaquén T.P.A. 23.555

JMS

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Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E. S. D.

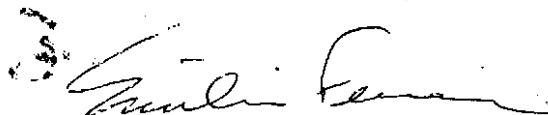
Poderdante : BOEHRINGER INGELHEIM INTERNATIONAL GMBH

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,

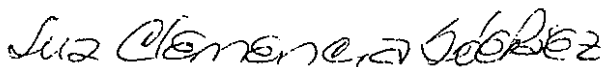


EMILIO FERRERO

T.P.A. N° 31.929

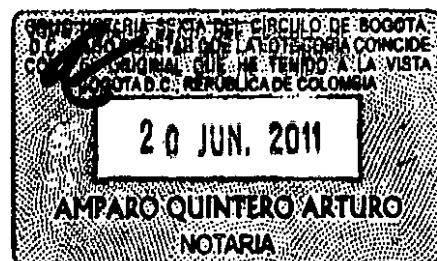
C.C. N° 438.019 de Usaquén

Acepto:


LUZ CLEMENCIA DE PAEZ

T.P.A. N° 23.555

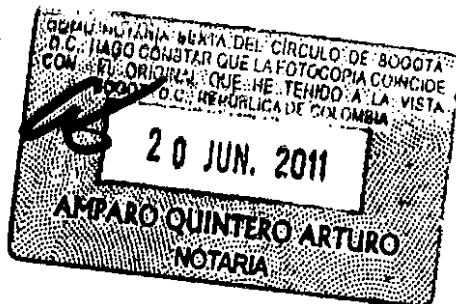
C.C. No. 35.456.344 de Usaquén



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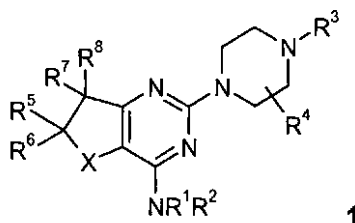
NOTARIA SEXTA DE BOGOTA
RECONOCIMIENTO Y PRESENTACION PERSONAL
Bogotá, D.C.
Ante el **03 NOV 2009**
NOTARIO SEXTO (E) DEL CIRCULO DE
BOGOTA D.C.
Compareció (eron) *Amirio Ferrero*
Suz Clemencia Soler
Amirio Ferrero
Quien(es) exhibió(aron) la(s) C.C.
Cc 7438019714
Cc 3545601273
Y declaró(aron) que la(s) 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.

Emilio Ferrero
Suz Clemencia Soler



REIVINDICACIONES

1. Compuestos de la fórmula 1,



en los que

X O, S, SO o SO₂;

R¹ H, alquilo C₁₋₆-, alquenilo C₂₋₆, alquinilo C₂₋₆, arilo C₆₋₁₀ o aril C₆₋₁₀-alquileo C₁₋₆, heteroarilo C₅₋₁₀-alquileo C₁₋₆;

R² H o un radical eventualmente mono- o polisustituido seleccionado del grupo compuesto por alquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆, un cicloalquilo C₃₋₁₀ mono- o bicyclico eventualmente puenteado una o varias veces, arilo C₆₋₁₀, un anillo C₃₋₁₀ heterocíclico aromático o no aromático, un anillo bicyclico y un arilo C₆₋₁₀ condensado con un heterociclo C₃₋₁₀;

o **NR¹R²** significan juntos un anillo heterocíclico que está eventualmente sustituido con uno o varios radicales seleccionados de alquilo C₁₋₄, alquenilo C₂₋₆, alquinilo C₂₋₆, alcohol C₁₋₆ lineal o ramificado y oxo;

R³ un radical mono- o polisustituido seleccionado del grupo compuesto por un anillo C₆₋₁₀ heterocíclico, cicloalquilo C₃₋₇, aril C₆₋₁₀-alquileo C₁₋₆, heteroaril C₅₋₁₀-alquileo C₁₋₆, un anillo bicyclico condensado que eventualmente puede contener 1-4 heteroátomos seleccionados, de modo independiente entre sí, de N, O, S,

o **R³** significa fenilo eventualmente sustituido;

o **R³** significa un grupo COR^{3.7}, COCH₂R^{3.8}, CONHR^{3.8} o SO₂R^{3.8};

en donde

R^{3.7} H, alquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆ o arilo C₆₋₁₀;

R^{3.8} alquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆, cicloalquilo C₃₋₇ o un radical seleccionado del grupo compuesto por arilo C₆₋₁₀, un anillo C₃₋₁₀ heterocíclico y un anillo bicyclico que eventualmente está sustituido con uno o varios radicales seleccionados del grupo compuesto por alquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆, halógeno, NR^{3.8.1}R^{3.8.2}, arilo C₆₋₁₀ y un anillo C₃₋₁₀ heterocíclico;

en donde

R^{3.8.1} H, alquilo C₁₋₆, alquenilo C₂₋₆ o alquinilo C₂₋₆;

R^{3.8.2} H, alquilo C₁₋₆, alquenilo C₂₋₆ o alquinilo C₂₋₆;

R⁴ H, alquilo C₁₋₄, alquenilo C₂₋₄, alquinilo C₂₋₄ u oxo;

R⁵ H, alquilo C₁₋₄, alquenilo C₂₋₄ o alquinilo C₂₋₄;

- R⁶** H, alquilo C₁₋₄, alquenilo C₂₋₄ o alquinilo C₂₋₄;
R⁷ H, alquilo C₁₋₄, alquenilo C₂₋₄, alquinilo C₂₋₄, arilo C₆₋₁₀ u OH;
R⁸ H, alquilo C₁₋₄, alquenilo C₂₋₄, alquinilo C₂₋₄, arilo C₆₋₁₀ u OH;
 o **R⁷** y **R⁸** forman juntos oxo;

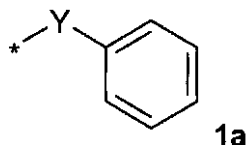
así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

2. Compuestos de la fórmula **1**, de acuerdo con la reivindicación **1**, en donde significan

- X** O, S, SO o SO₂;
R¹ H, alquilo C₁₋₆, arilo C₆₋₁₀, aril C₆₋₁₀-alquilenilo C₁₋₆, heteroaril C₅₋₁₀-alquilenilo C₁₋₆,
R² H o alquilo C₁₋₆, que puede estar sustituido eventualmente con uno o varios radicales seleccionados del grupo compuesto por haloalquilo C₁₋₆, CN, OR^{2.1}, NR^{2.1}R^{2.2}, COOR^{2.1}, CONR^{2.1}R^{2.2}, cicloalquilo C₃₋₇ eventualmente sustituido con alquilo C₁₋₄ o con oxo, heterociclo aromático o no aromático eventualmente sustituido con alquilo C₁₋₄, con oxo, con OH o con halógeno, arilo C₆₋₁₀ eventualmente sustituido con alquilo C₁₋₄ o con oxo y arilo C₆₋₁₀ condensado con un heterociclo C₅₋₆, en donde este sistema de anillos condensado puede estar sustituido eventualmente con alquilo C₁₋₄ o con oxo,
 en donde
R^{2.1} H o alquilo C₁₋₆; que eventualmente está sustituido con un cicloalquilo C₃₋₇, heterociclo C₃₋₁₀ o arilo C₆₋₁₀ eventualmente sustituidos,
R^{2.2} H o alquilo C₁₋₆;
 o **R²** un radical seleccionado de cicloalquilo C₃₋₁₀ eventualmente puenteado una o varias veces o un cicloalquilo C₃₋₁₀ que eventualmente puede estar condensado con un anillo arilo C₆₋₁₀ que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por alquilo C₁₋₆, OH, CH₂OR^{2.3}, COOR^{2.3}, COR^{2.3}, CONR^{2.3}R^{2.4}, O-alquilo C₁₋₆, O-aralquilo C₇₋₁₁, NR^{2.3}R^{2.4} y NHCOR^{2.5},
 en donde
R^{2.3} H o un heterociclo o un alquilo C₁₋₆, que eventualmente puede estar sustituido con un radical seleccionado de cicloalquilo C₃₋₇, heterociclo C₃₋₁₀ y arilo C₆₋₁₀, en donde este radical puede estar eventualmente sustituido con uno o varios radicales del grupo alquilo C₁₋₆, halógeno, OH y O-alquilo C₁₋₆;
 en donde
R^{2.4} H o alquilo C₁₋₆;

$R^{2.5}$ un radical seleccionado del grupo compuesto por cicloalquilo C_{3-7} , un anillo C_{3-10} heterocíclico y alquilo C_{1-6} , que puede estar eventualmente sustituido con OH;

- o R^2 es un grupo de la fórmula **1a**,



Y es alquileo C_{1-6} , eventualmente sustituido con uno o dos $R^{2.7}$ en donde $R^{2.7}$ están seleccionados, de modo independiente entre sí, de alquilo C_{1-6} , COOH, CONH₂, OR^{2.1} y COOR^{2.1}; o $R^{2.7}$ forma junto con uno o dos átomos de carbono de Y un anillo carbocíclico con 3 átomos de carbono,

- o R^2 arilo C_{6-10} , que puede estar eventualmente sustituido con uno o varios radicales seleccionados, de modo independiente entre sí, del grupo compuesto por alquilo C_{1-6} , haloalquilo C_{1-6} , alquenilo C_{2-6} , alquinilo C_{2-6} , CN, halógeno, OR^{2.8}, COOR^{2.8}, COR^{2.10}, NHCOMe, CONR^{2.3}R^{2.4}, un radical alquileo C_{1-4} sustituido con NR^{2.1}R^{2.2}, NR^{2.1}R^{2.2},
- o R^2 airlo C_{6-10} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados, de modo independiente entre sí, del grupo compuesto por aril C_{6-10} -alquileo C_{1-6} , heteroaril C_{5-10} -alquileo C_{1-6} , cicloalquilo C_{3-7} , un cicloalquil C_{3-7} -alquileo C_{1-4} , arilo C_{6-10} y un anillo C_{3-10} heterocíclico, en donde estos radicales pueden estar eventualmente sustituidos con uno o varios radicales seleccionados de alquilo C_{1-6} , haloalquilo C_{1-6} , COOR^{2.8}, CN, halógeno, OR^{2.8}, NHCOR^{2.8}, oxo, un heterociclo C_{3-10} , un cicloalquil C_{3-7} -alquileo C_{1-4} , un heterociclo C_{5-10} -alquileo C_{1-4} y un NR^{2.1}R^{2.2}-alquileo C_{1-4} ,

en donde

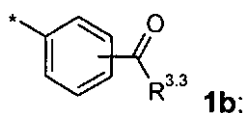
$R^{2.8}$ H, alquilo C_{1-6} , arilo C_{6-10} , un radical NR^{2.1}R^{2.2}-alquileo C_{1-4} ;

$R^{2.10}$ NHR^{2.10.1} o un anillo C_{3-10} heterocíclico que eventualmente puede estar sustituido con alquilo C_{1-4} ;

$R^{2.10.1}$ H, cicloalquilo C_{3-7} , alquenilo C_{2-6} , alquinilo C_{2-6} , alquilo C_{1-6} o alquil C_{1-6} -O-alquilo C_{1-4}

- o R^2 significa alquilo C_{6-10} , en el que está condensado un heterociclo C_{3-10} aromático o no aromático;
- o R^2 significa un anillo C_{3-10} heterocíclico aromático o no aromático que puede estar eventualmente sustituido con uno o varios radicales seleccionados del grupo compuesto por halógeno, OH, oxo-, CN, alquilo C_{1-6} , alcohol C_{1-6} , aril C_{6-10} -alquileo C_{1-6} , heteroaril C_{5-10} -alquileo C_{1-6} , COR^{2.11}, cicloalquil C_{3-7} -alquileo C_{1-4} y heterociclo C_{3-10} -alquileo C_{1-4} ;
- en donde

- $R^{2.11}$ un radical seleccionado del grupo compuesto por heterociclo C_{3-10} -alquileo C_{1-4} , cicloalquilo C_{3-7} y un anillo C_{3-10} heterocíclico aromático o no aromático,
que eventualmente puede estar sustituido con alquilo C_{1-6} , que a su vez puede estar eventualmente sustituido con OH, CH_2OH , OMe, NH_2 , un heterociclo C_{3-10} o $NHCOO$ - t Bu;
- o R^2 un radical seleccionado del grupo compuesto por alqueno C_{2-6} o un anillo bicíclico que puede estar eventualmente sustituido con metilo;
- o NR^1R^2 un anillo heterocíclico que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo alquilo C_{1-4} , OH y alcohol C_{1-4} ;
- R^3 un radical seleccionado del grupo compuesto por un anillo C_{3-10} heterocíclico, un cicloalquilo C_{3-7} , un sistema de anillos bicíclicos, condensados, aromáticos o no aromáticos que contiene 1 a 4 heteroátomos seleccionados de S, N, O, aril C_{6-10} -alquileo C_{1-6} , heteroaril C_{5-10} -alquileo C_{1-6} y CH_2 -benzo[1, 3]dioxolilo, que eventualmente puede estar sustituido con uno o varios radicales seleccionados de OH, halógeno, alquilo C_{1-6} , O-alquilo C_{1-6} , haloalquilo C_{1-6} y $CO-R^{3.1}$,
- o R^3 fenilo que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por **alquilo** C_{1-6} , alqueno C_{2-6} , alquino C_{2-6} , haloalquilo C_{1-4} , alquien $C_{1-6}-NR^{3.1}R^{3.2}$, CN, $COOR^{3.1}$, $CONR^{3.1}R^{3.2}$, $NR^{3.1}R^{3.2}$, $NHCOR^{3.1}$, CF_3 , $OR^{3.1}$, halógeno, $NHCOR^{3.1}$, NO_2 , $SO_2NR^{3.1}R^{3.2}$ y alquien $C_{1-6}-NHCOR^{3.1}$;
- en donde
- $R^{3.1}$ H, alquilo C_{1-6} , alqueno C_{2-6} o alquino C_{2-6} , heterociclo C_{3-10} mono- o bicíclico eventualmente puenteado o radical heterociclo C_{3-10} -alquileo C_{1-4} ;
- $R^{3.2}$ H, alquilo C_{1-6} , alqueno C_{2-6} o alquino C_{2-6} ;
- o R^3 un grupo de la fórmula 1b



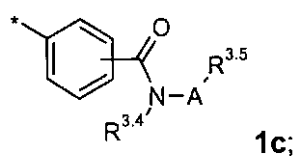
en los que

- $R^{3.3}$ un radical seleccionado del grupo compuesto por un anillo C_{3-10} heterocíclico que puede estar eventualmente sustituido con uno o varios radicales seleccionados del grupo compuesto por alquilo C_{1-6} , oxo, $COR^{3.3.1}$, $COR^{3.3.2}$, alquien $C_{1-6}-R^{3.3.2}$, CH_2CO -pirrolidina y un anillo C_{3-10} heterocíclico, en donde un átomo de azufre contenido en el anillo heterocíclico puede estar presente eventualmente como óxido o dióxido;

en donde

- $R^{3.3.1}$ alquilo C_{1-6} ;
- $R^{3.3.2}$ NH_2 , NH (alquilo C_{1-6}), N (alquilo C_{1-6}) $_2$;
- o $R^{3.3}$ anillo bicíclico o anillo espiro heterocíclico;

- o R^3 un grupo de la fórmula **1c**;



en los que

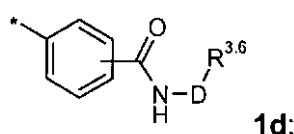
A enlace o alquilo C_{1-6} , que puede estar eventualmente sustituido con oxo o NMe_2 ;

$R^{3.4}$ H o alquilo C_{1-6} ;

$R^{3.5}$ un radical seleccionado del grupo compuesto por alquilo C_{1-6} , que puede estar eventualmente sustituido con uno o varios radicales seleccionados de cicloalquilo C_{3-7} , arilo C_{6-10} y un heterociclo C_{3-10} , en donde este radical puede estar eventualmente sustituido con un radical seleccionado del grupo OH, oxo, alquilo C_{1-6} , O-alquilo C_{1-6} , haloalquilo C_{1-6} , o un radical seleccionado de un anillo heterocíclico C_{3-10} y un anillo bicíclico que puede estar eventualmente sustituido con uno o varios radicales seleccionados, de modo independiente entre sí, del grupo compuesto por oxo, alquilo C_{1-6} , OH, arilo C_{6-10} , un anillo C_{3-10} heterocíclico, alquilen $C_{1-6}-R^{3.5.1}$, O-alquilen $C_{1-6}-R^{3.5.1}$ y NH-alquilen $C_{1-6}-R^{3.5.1}$,

$R^{3.5.1}$ un radical seleccionado del grupo compuesto por arilo C_{6-10} , anillo C_{3-10} heterocíclico que eventualmente puede estar sustituido con alquilo C_{1-6} ;

- o R^3 significa un grupo de la fórmula **1d**;



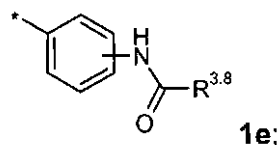
en los que

D alquilenilo C_{2-4} ; un radical cicloalquilo C_{3-10} bicíclico eventualmente puentado que puede estar eventualmente sustituido con de uno o varios radicales seleccionados de alquilo C_{1-6} , halógeno, OH, haloalquilo C_{1-6} y O-alquilo C_{1-6} ,

$R^{3.6}$ piridinilo;

- o R^3 significa un radical seleccionado del grupo de $COR^{3.7}$, $COCH_2R^{3.8}$, $CONHR^{3.8}$, $SO_2R^{3.8}$ y un heterociclo condensado con un radical arilo C_{6-10} que eventualmente puede estar sustituido con metilo,

- o R^3 un grupo de la fórmula **1e**;



$R^{3.7}$ H, alquilo C_{1-6} , arilo C_{6-10} ;

$R^{3.8}$ radical seleccionado del grupo de alquilo C_{1-6} , cicloalquilo C_{3-7} o un radical seleccionado del grupo compuesto por arilo C_{6-10} , anillo heterocíclico C_{3-10} y un anillo bicíclico que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por alquilo C_{1-6} , halógeno, $NR^{3.8.1}R^{3.8.2}$, arilo C_{6-10} y un anillo heterocíclico C_{3-10} ;

$R^{3.8.1}$ H o alquilo C_{1-6} ;

$R^{3.8.2}$ H o alquilo C_{1-6} ;

R^4 H, alquilo C_{1-4} u oxo;

R^5 H o alquilo C_{1-4} ;

R^6 H o alquilo C_{1-4} ;

R^7 H, alquilo C_{1-4} , arilo C_{6-10} u OH;

R^8 H, alquilo C_{1-4} , arilo C_{6-10} u OH;

o R^7 y R^8 forman juntos oxo;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

3. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 ó 2, en donde significan

X O, S, SO o SO_2 ;

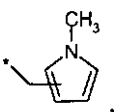
R^1 H, metilo, etilo o propilo;

R^2 H o alquilo C_{1-6} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por CF_3 , CN, OH, NMe_2 , OMe, COOH y $CONMe_2$,

o R^2 alquilo C_{1-6} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados de ciclopropilo, ciclopentilo, ciclohexilo, fenilo, pirrolidinilo, imidazolidinilo, pirazolilo, imidazolilo y piridinilo, que eventualmente puede estar sustituido con metilo u oxo;

o R^2 cicloalquilo C_{3-7} , que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por metilo, $OR^{2.3}$, $CH_2OR^{2.3}$, COOH, $CONR^{2.3}R^{2.4}$, $CONH^tBu$, O-bencilo, $NR^{2.3}R^{2.4}$ y $NHCOR^{2.5}$,

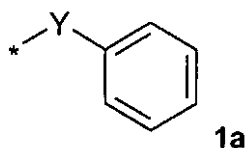
en donde

$R^{2.3}$ H o metilo, ,

$R^{2.4}$ H o metilo;

$R^{2.5}$ $CH_2C(CH_3)_3$, $CH_2C(CH_3)_2(CH_2OH)$, ciclopentilo, tetrahidrofuranilo, pirrolilo, pirazolilo, imidazolilo o isoxazolilo;

- o R^2 significa un grupo de la fórmula **1a**,



Y alquileo C_{1-4} , eventualmente sustituido con uno o dos $R^{2.7}$

$R^{2.7}$ de modo independiente entre sí, alquilo C_{1-4} , $COOH$, $CONH_2$; o $R^{2.7}$ forma junto con uno o dos átomos de carbono de Y un anillo carbocíclico con 3 átomos de carbono,

- o R^2 arilo C_{6-10} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados, de modo independiente entre sí, del grupo compuesto por metilo, *tert*-butilo, CN , F , Cl , Br , OH , OMe , OEt , *O*-fenilo, $COOH$, $COOMe$, $COR^{2.10}$, $NHCOMe$ y alquileo C_{1-4} sustituido con morfolina,

en donde

$R^{2.10}$ NH_2 , $NHMe$, NH^iPr , NH -ciclopropilo, $NHCH_2CH_2OMe$ o un anillo C_{3-10} heterocíclico no aromático que puede contener uno, dos o tres heteroátomos seleccionados del grupo oxígeno y nitrógeno;

- o R^2 arilo C_{6-10} , que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por fenilo y un anillo C_{3-10} heterocíclico, que eventualmente puede estar sustituido con uno o varios radicales del grupo compuesto por metilo, *tert*-butilo, $COOH$, $COOMe$, CN , F , Cl , Br , OH , OMe , OEt y $NHCOMe$, oxo;

- o R^2 un anillo C_{5-10} heterocíclico no aromático eventualmente sustituido con un radical seleccionado del grupo compuesto por bencilo y $COR^{2.11}$;

en donde significan

$R^{2.11}$ un radical seleccionado del grupo compuesto por ciclopentilo, tetrahidrofurano, furano, piridilo, pirrolilo, pirazolilo, imidazolilo, que eventualmente puede estar sustituido con uno o dos grupos metilo, o con uno o varios radicales del grupo $CH_2C(CH_3)_3$, $C(CH_3)_2(CH_2OH)$, CH_2OMe , $C(CH_3)_2NH_2$ y $C(CH_3)_2NHCOO^iBu$;

- o R^2 un radical seleccionado del grupo compuesto por alqueno C_{2-6} , indanilo, 1, 2, 3, 4-tetrahidro-naftalilo y 8-metil-8-aza-biciclo[3.2.1]octano;

- o NR^1R^2 un radical seleccionado del grupo compuesto por pirrolidinilo, piperidinilo, piperazinilo y morfolinilo, que eventualmente puede estar sustituido con metilo;

R^3 un radical seleccionado del grupo compuesto por piridinilo, pirimidina, bencilo y CH_2 -Benzo[1, 3]dioxolilo;

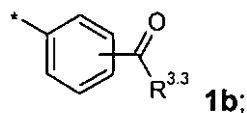
- o R^3 fenilo, que eventualmente puede estar sustituido con uno, dos o tres radicales seleccionados del grupo compuesto por metilo, CH_2NH_2 , CN , $COOH$, $CONH_2$, CF_3 , OH , F , Cl , Br , OMe , $NHCOMe$, $NR^{3.1}COR^{3.2}$, $CONR^{3.1}R^{3.2}$, NO_2 , $SONMe_2$ y $CH_2NHCOMe$;

en donde

$R^{3.1}$ H, alquilo C_{1-6} o un heterociclo C_{3-10} mono- o bicíclico eventualmente puenteado;

$R^{3.2}$ H o alquilo C_{1-6} ;

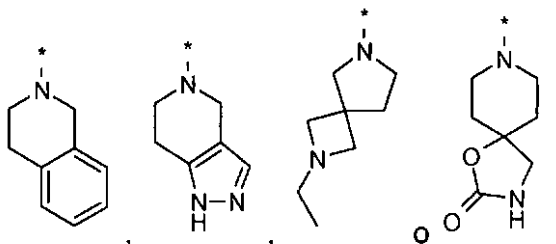
o R^3 un grupo de la fórmula **1b**



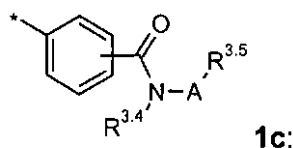
en donde significan

$R^{3.3}$ un radical seleccionado del grupo compuesto por piperidinilo, piperazinilo, azepanilo, que eventualmente puede estar sustituido con uno o varios radicales seleccionados, de modo independiente entre sí, del grupo compuesto por metilo, oxo, $COCH_3$, $CONH_2$, CH_2Net_2 , $CH_2CH_2NMe_2$, CH_2CO -pirrolidina, piridinilo, isotiazolidinil-1,1-dióxido y tiazolidinil-1,1-dióxido;

o $R^{3.3}$ significa un radical de la fórmula



o R^3 significa un grupo de la fórmula **1c**;



en los que

A enlace o alquilo C_{1-4} , que puede estar eventualmente sustituido con oxo o NMe_2 ;

$R^{3.4}$ H o metilo;

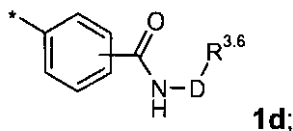
$R^{3.5}$ un radical seleccionado del grupo compuesto por pirrolidinilo, piperidinilo, piperazinilo, morfolinilo, ciclohexilo, imidazolilo, pirazolilo, fenilo, piridinilo, bencimidazolilo, imidazolidin-2-ona, pirrolidin-2-ona, pirrolidin-3-ona, tetrahidro-tiofen-1,1-dióxido y 1-aza-biciclo[2.2.2]octano, que eventualmente puede estar sustituido con uno o varios radicales seleccionados, de modo independiente entre sí, del grupo compuesto por metilo, etilo, OH, fenilo, piridinilo, pirazolilo, pirrolidinilo, $(CH_2)_6-R^{3.5.1}$, $O-(CH_2)_6-R^{3.5.1}$ y $NH-(CH_2)_6-R^{3.5.1}$;

en donde

o 0, 1 ó 2

$R^{3.5.1}$ un radical seleccionado del grupo compuesto por fenilo, pirrolidinilo, piperidinilo e imidazolidin-2-ona, que eventualmente puede estar sustituido con metilo;

o R^3 significa un grupo de la fórmula **1d**;

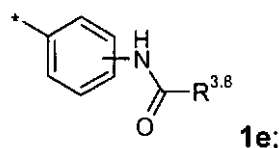


en los que

D alquinilo C_{2-4} ;

$R^{3.6}$ piridinilo;

o R^3 significa un grupo $COR^{3.7}$, $COCH_2R^{3.8}$, $CONHR^{3.8}$, $SO_2R^{3.8}$ o un grupo de la fórmula **1e**;



en los que

$R^{3.7}$ H, metilo, fenilo;

$R^{3.8}$ un radical seleccionado del grupo compuesto por *iso*-propilo, ciclopropilo, ciclopentilo, ciclohexilo, pirrolidinilo, pirrolidin-2-ona, furanilo y azabicclo[2.2.2]octanilo o un radical seleccionado del grupo compuesto por piperidinilo, pirazolilo, imidiazolilo, isoxazolilo, piridinilo, fenilo, bencilo, que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por metilo, cloro, NH_2 , NMe_2 , fenilo y morfolinilo;

R^4 H, metilo u oxo;

R^5 H o metilo;

R^6 H o metilo;

R^7 H, metilo u OH; con preferencia H o metilo;

R^8 H, metilo u OH; con preferencia H o metilo;

o R^7 y R^8 forman juntos oxo;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

4. Compuestos de la fórmula **1**, de acuerdo con una de las reivindicaciones 1 a 3, en donde significa

R^1 H;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

5. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 4, en donde significa

X SO;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

6. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 5, en donde significan

R^5, R^6, R^7, R^8 H;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

7. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 6, en donde significa

R^4 H;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

8. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 7, en donde significan

R^2 arilo C_{6-10} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados, de modo independiente entre sí, del grupo compuesto por alquilo C_{1-6} , haloalquilo C_{1-4} , alqueno C_{2-6} , alquino C_{2-6} , CN, halógeno, $OR^{2.8}$, $COOR^{2.8}$, $COR^{2.10}$, $NR^{2.8}R^{2.9}$, $NHCOR^{2.8}$, $SR^{2.8}$, $SOR^{2.8}$, $SO_2R^{2.8}$ y $SO_2NR^{2.8}R^{2.9}$

- o R^2 arilo C_{6-10} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por aril C_{6-10} -alquileo C_{1-6} , heteroaril C_{5-10} -alquileo C_{1-6} , arilo C_{6-10} y un anillo C_{3-10} heterocíclico, que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por alquilo C_{1-6} , haloalquilo C_{1-6} , $COOR^{2,8}$, CN , halógeno, $OR^{2,8}$, $NHCOR^{2,8}$, oxo, un cicloalquil C_{3-7} -alquileo C_{1-4} , un heterociclo-alquileo C_{1-4} y un $NR^{2,1}R^{2,2}$ -alquileo C_{1-4} , en donde
 - $R^{2,8}$ H, alquilo C_{1-6} , alquenilo C_{2-6} , alquinilo C_{2-6} , un $NR^{2,1}R^{2,2}$ -alquileo C_{1-4} o arilo C_{6-10} ;
 - $R^{2,9}$ H, alquilo C_{1-6} , alquenilo C_{2-6} o alquinilo C_{2-6} ;
 - $R^{2,10}$ $NHR^{2,10,1}$, alquilen C_{1-6} -O-alquilo C_{1-4} , o un anillo C_{3-10} heterocíclico, que eventualmente puede estar sustituido con alquilo C_{1-4} ;
 - $R^{2,10,1}$ H, alquilo C_{1-6} , alquenilo C_{2-6} , alquinilo C_{2-6} o cicloalquilo C_{3-7} ;
- o R^2 significa alquilo C_{6-10} , en el que está condensado un heterociclo C_{3-10} aromático o no aromático;
- o R^2 arilo C_{6-10} , que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por arilo C_{6-10} y un anillo C_{3-10} heterocíclico, que eventualmente puede estar sustituido con uno o varios radicales del grupo alquilo C_{1-6} , alquenilo C_{2-6} , alquinilo C_{2-6} , haloalquilo C_{1-4} , CN , halógeno, $OR^{2,8}$, $COOR^{2,8}$, $COR^{2,10}$, $NR^{2,8}R^{2,9}$, $NHCOR^{2,8}$, $SR^{2,8}$, $SOR^{2,8}$, $SO_2R^{2,8}$, $SO_2NR^{2,8}R^{2,9}$ y oxo;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

9. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 8, en donde significan

- R^2 arilo C_{6-10} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por alquilo C_{1-4} , CN , halógeno, $OR^{2,8}$, $COOR^{2,8}$, $COR^{2,10}$ y $NHCOMe$
 - $R^{2,8}$ alquilo C_{1-4} o arilo C_{6-10} ;
 - $R^{2,10}$ $NHR^{2,10,1}$, morfolinilo, metil-piperazinilo;
 - $R^{2,10,1}$ H, ciclopropilo o alquilo C_{1-4} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados de O-alquilo C_{1-4} , OH o arilo C_{6-10} ;
- o R^2 arilo C_{6-10} , que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por fenilo y un anillo C_{3-10} heterocíclico, que

eventualmente puede estar sustituido con alquilo C_{1-4} , $COOR^{2,8}$, CN, halógeno, $OR^{2,8}$, $NHCOMe$ y oxo;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

10. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 7, en donde significan

R^2 un radical seleccionado del grupo compuesto por alquilo C_{1-6} , alquenilo C_{2-6} y alquinilo C_{2-6} , que puede estar eventualmente sustituido con un radical seleccionado del grupo compuesto por haloalquilo C_{1-6} , CN, $OR^{2,1}$, $NR^{2,1}R^{2,2}$, $NHCOR^{2,1}$, $SR^{2,1}$, $SOR^{2,1}$, $SO_2R^{2,1}$, $SO_2NR^{2,1}R^{2,2}$, $COOR^{2,1}$ y $CONR^{2,1}R^{2,2}$, que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por cicloalquilo C_{3-7} , arilo C_{6-10} y un anillo C_{3-10} heterocíclico que eventualmente puede estar sustituido, a su vez, con uno o varios radicales seleccionados del grupo de alquilo C_{1-6} , alquenilo C_{2-6} , alquinilo C_{2-6} u oxo;

en donde significan

$R^{2,1}$ H, alquilo C_{1-6} , alquenilo C_{2-6} o alquinilo C_{2-6} ;

$R^{2,2}$ H, alquilo C_{1-6} , alquenilo C_{2-6} o alquinilo C_{2-6} ;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

11. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 7 y 10, en donde significan

R^2 alquilo C_{1-6} , que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por haloalquilo C_{1-4} , CN, $OR^{2,1}$, $NR^{2,1}R^{2,2}$, $COOR^{2,1}$ y $CONR^{2,1}R^{2,2}$ o que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por cicloalquilo C_{3-7} , arilo C_{6-10} y un anillo C_{3-10} heterocíclico aromático que puede estar eventualmente sustituido, a su vez, con metilo u oxo;

en los que

$R^{2,1}$ H o alquilo C_{1-4} ;

$R^{2,2}$ H o alquilo C_{1-4} ;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

12. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 9, en donde significan

R² arilo C₆₋₁₀, que eventualmente puede estar sustituido en posición meta con uno o varios radicales seleccionados, de modo independiente entre sí, del grupo compuesto por alquilo C₁₋₄, CN, halógeno, OR^{2.8}, COOR^{2.8}, COR^{2.10} y NHCOMe

en donde

R^{2.8} alquilo C₁₋₄ o arilo C₆₋₁₀;

R^{2.10} NHR^{2.10.1}, morfolinilo, metil-piperazinilo;

R^{2.10.1} H, ciclopropilo o alquilo C₁₋₄, en donde el alquilo C₁₋₄ puede estar eventualmente sustituido con uno o varios radicales seleccionados del grupo O-alquilo C₁₋₄, OH y arilo C₆₋₁₀;

R² NH(R^{2.10.1}), ciclohexilo

o **NR¹R²** un anillo C₅₋₆ heterocíclico seleccionado del grupo compuesto por pirrolidina y piperazina, que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por alquilo C₁₋₄, OH y C₁₋₄-alcohol

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

13. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 9 y 12, en donde significan

R³ fenilo, que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por alquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₄, alquilen C₁₋₆--NR^{3.1}R^{3.2}, CN, halógeno, OR^{3.1}, COOR^{3.1}, CONR^{3.1}R^{3.2}, NR^{3.1}R^{3.2}, NHCOR^{3.1}, NO₂, SR^{3.1}, SOR^{3.1}, SO₂R^{3.1}, SO₂NR^{3.1}R^{3.2} y alquilen C₁₋₆--NHCOR^{3.1};

en los que

R^{3.1} H, alquilo C₁₋₆, alquenilo C₂₋₆ o alquinilo C₂₋₆;

R^{3.2} H, alquilo C₁₋₆, alquenilo C₂₋₆ o alquinilo C₂₋₆;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido

bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

14. Compuestos de la fórmula **1**, de acuerdo con una de las reivindicaciones 1 a 9 y 12 a 13, en donde significan

R³ fenilo que puede estar eventualmente sustituido en posición para con un radical seleccionado del grupo compuesto por alquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₄, alquilen C₁₋₆-NR^{3.1}R^{3.2}, CN, halógeno, OR^{3.1}, COOR^{3.1}, CONR^{3.1}R^{3.2}, NR^{3.1}R^{3.2}, NHCOR^{3.1}, NO₂, SR^{3.1}, SOR^{3.1}, SO₂R^{3.1}, SO₂NR^{3.1}R^{3.2} y alquilen C₁₋₆-NHCOR^{3.1},

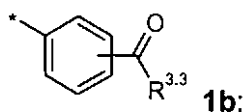
en los que

R^{3.1} H, alquilo C₁₋₆, alquenilo C₂₋₆ o alquinilo C₂₋₆;

R^{3.2} H, alquilo C₁₋₆, alquenilo C₂₋₆ o alquinilo C₂₋₆;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

15. Compuestos de la fórmula **1**, de acuerdo con una de las reivindicaciones 1 a 9 y 12 a 14, en donde **R³** es un grupo de la fórmula **1b**



en los que

R^{3.3} un anillo C₃₋₁₀ heterocíclico que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por alquilo C₁₋₆, oxo, COR^{3.3.1}, COR^{3.3.2}, alquilen C₁₋₆-R^{3.3.2}, CH₂CO-pirrolidina y un anillo C₃₋₁₀ heterocíclico, en donde puede haber un átomo de azufre contenido en el anillo C₃₋₁₀ heterocíclico también como óxido o dióxido;

en donde

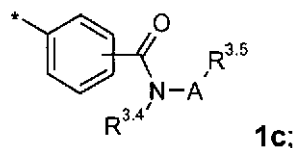
R^{3.3.1} alquilo C₁₋₆;

R^{3.3.2} NH₂, NH(alquilo C₁₋₆) o N(alquilo C₁₋₆)₂;

o **R^{3.3}** un anillo bicíclico o un anillo espiro heterocíclico;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

16. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 9 y 12 a 15, en donde R^3 es un grupo de la fórmula 1c;



en los que

A enlace o alquilo C_{1-6} , que puede estar eventualmente sustituido con oxo o NMe_2 ;

$R^{3.4}$ H o alquilo C_{1-6} ;

$R^{3.5}$ un radical seleccionado del grupo compuesto por alquilo C_{1-6} , que eventualmente puede estar sustituido con un radical seleccionado del grupo compuesto por cicloalquilo C_{3-7} , arilo C_{6-10} y un heterociclo C_{5-10} , que también puede estar eventualmente sustituido con uno o varios radicales seleccionados del grupo de halógeno, OH, oxo, alquilo C_{1-6} , O-alquilo C_{1-6} , haloalquilo C_{1-6} , un anillo C_{3-10} heterocíclico y un anillo bicíclico, en donde estos radicales pueden estar en cada caso eventualmente sustituidos con uno o varios radicales seleccionados del grupo compuesto por oxo, alquilo C_{1-6} , OH, arilo C_{6-10} , un radical heterocíclico, alquilen $C_{1-6}-R^{3.5.1}$, O-alquilen $C_{1-6}-R^{3.5.1}$ y NH-alquilen $C_{1-6}-R^{3.5.1}$,

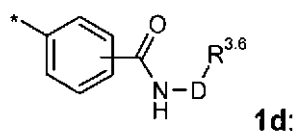
en donde

$R^{3.5.1}$ un radical seleccionado del grupo compuesto por arilo C_{6-10} y anillo C_{3-10} heterocíclico que eventualmente puede estar sustituido con alquilo C_{1-6} ;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

17. Compuestos de la fórmula 1 de acuerdo con una de las reivindicaciones 1 a 9 y 12 a 16, en donde

R^3 es un grupo de la fórmula 1d;



en donde significan

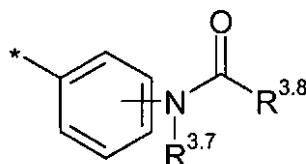
D alquinilo C_{2-4} ;

$R^{3.6}$ piridinilo;

así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

18. Compuestos de la fórmula 1, de acuerdo con una de las reivindicaciones 1 a 9 y 12 a 17, en donde significa

R³ un grupo de la fórmula 1e;



en donde significan

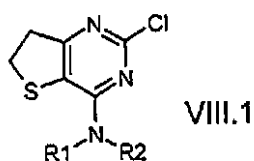
R^{3.7} H, halógeno, alquilo C₁₋₆, alquenilo C₂₋₆; alquinilo C₂₋₆ o haloalquilo C₁₋₆;

R^{3.8} H, OH, halógeno o un radical seleccionado del grupo compuesto por alquilo C₁₋₆, alquenilo C₂₋₆; alquinilo C₂₋₆, haloalquilo C₁₋₆, O-alquilo C₁₋₆, arilo C₆₋₁₀, un anillo C₃₋₁₀ heterocíclico y un anillo bicíclico, que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo de halógeno, alquilo C₁₋₆, OH, haloalquilo C₁₋₆ y O-alquilo C₁₋₆.

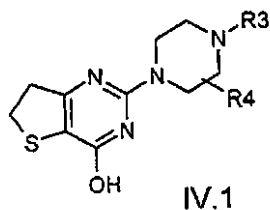
así como sus sales de adición de ácidos farmacológicamente tolerables con un ácido orgánico o inorgánico seleccionado del grupo que consiste de ácido succínico, ácido bromhídrico, ácido acético, ácido fumárico, ácido maléico, ácido metanosulfónico, ácido láctico, ácido fosfórico, ácido clorhídrico, ácido sulfúrico, ácido tartárico y ácido cítrico.

19. Productos intermediarios seleccionados del grupo compuesto por:

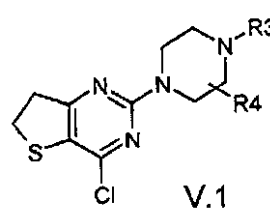
- compuestos de acuerdo con la fórmula VIII.1



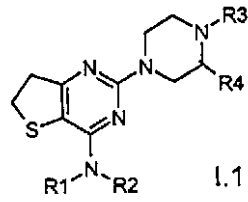
- compuestos de acuerdo con la fórmula IV.1 y V.1



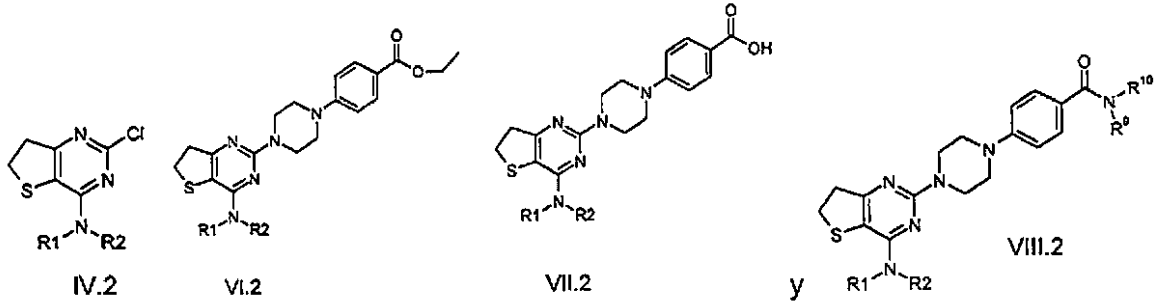
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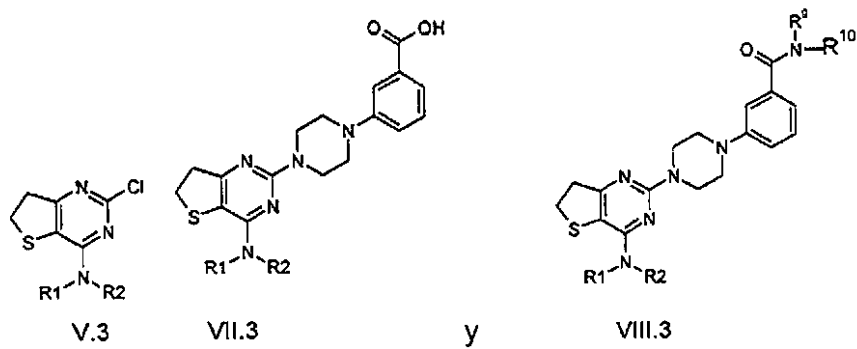
- compuestos de acuerdo con la fórmula I.1



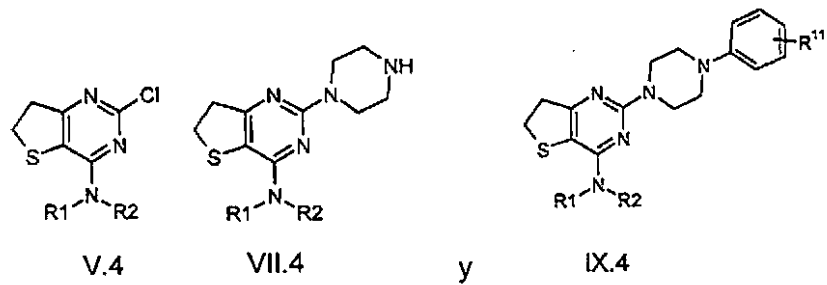
- compuestos de acuerdo con la fórmula IV.2, VI.2, VII.2 y VIII.2



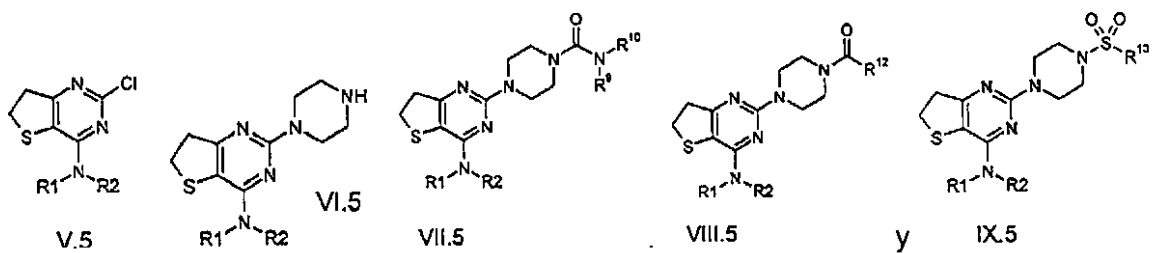
- compuestos de acuerdo con la fórmula V.3, VII.3 y VIII.3



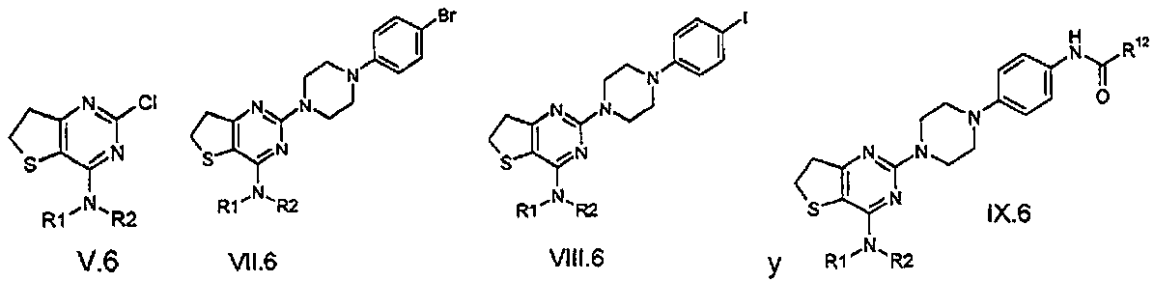
- compuestos de acuerdo con la fórmula V.4, VII.4 y IX.4



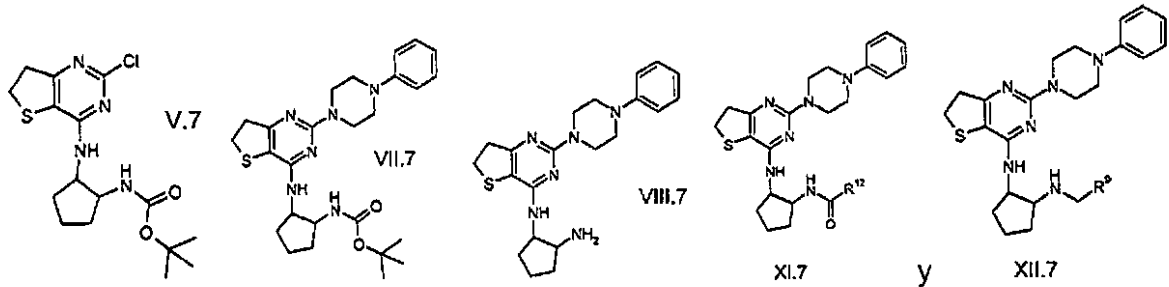
- compuestos de acuerdo con la fórmula V.5, VI.5, VII.5, VIII.5 y IX.5



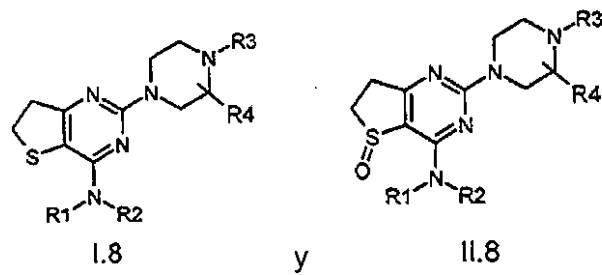
- compuestos de acuerdo con la fórmula V.6, VII.6, VIII.6 y IX.6



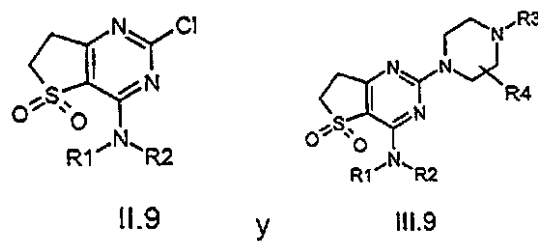
- compuestos de acuerdo con la fórmula V.7, VII.7, VIII.7, XI.7 y XII.7



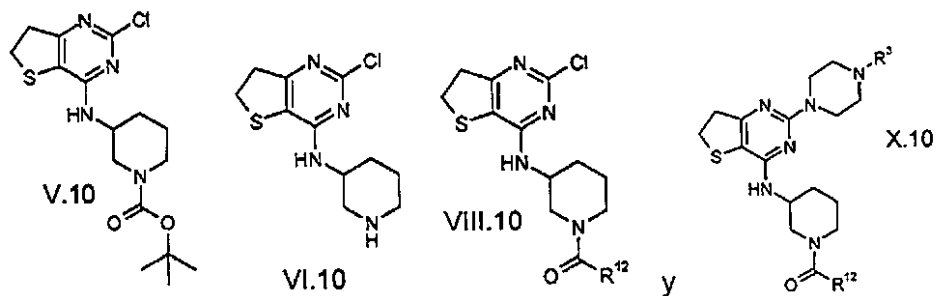
- compuestos de acuerdo con la fórmula I.8 y II.8



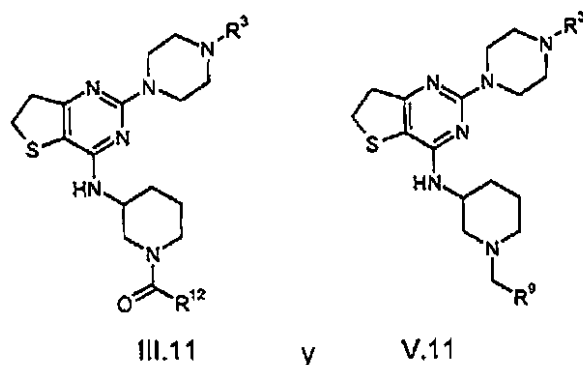
- compuestos de acuerdo con la fórmula II.9 y III.9



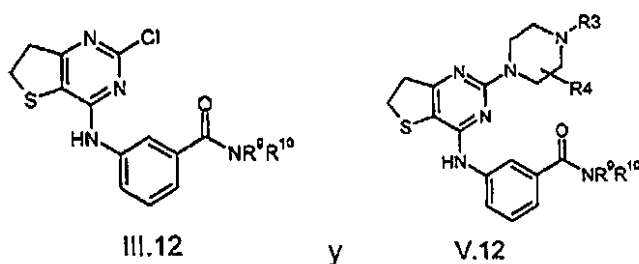
- compuestos de acuerdo con la fórmula V.10, VI.10, VIII.10 y X.10



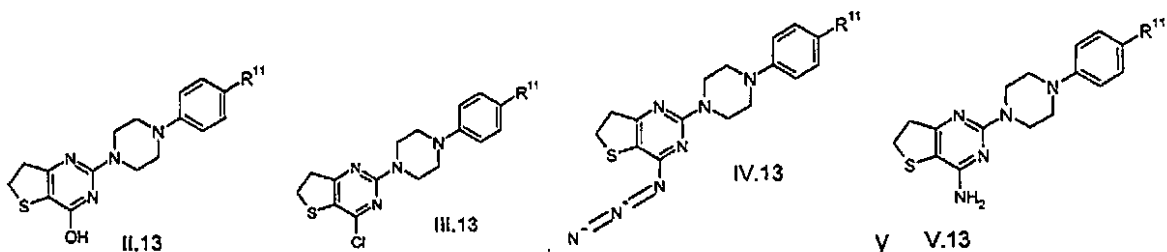
- compuestos de acuerdo con la fórmula III.11 y V.11



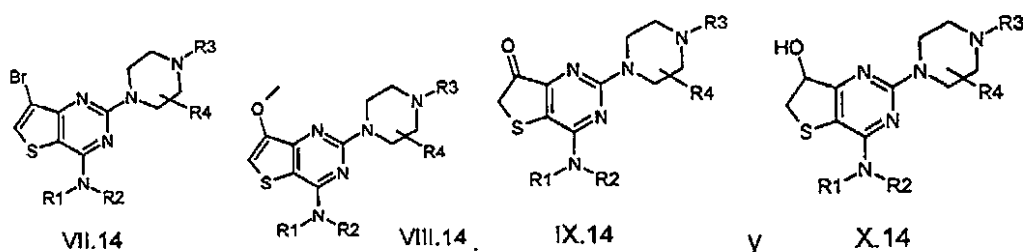
- compuestos de acuerdo con la fórmula III.12 y V.12



- compuestos de acuerdo con la fórmula II.13, III.13 y IV.13 y V.13



- compuestos de acuerdo con la fórmula VII.14, VIII.14, IX.14 y X.14



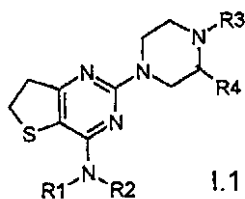
en donde R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 y X tienen los significados definidos en las reivindicaciones 1 a 18,

y en donde R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 y X tienen los significados antes definidos y en donde R^9 , R^{10} , R^{11} , R^{12} son, en cada caso de modo independiente entre sí, un radical seleccionado del grupo compuesto por H, halógeno, alquilo C_{1-6} , haloalquilo C_{1-6} , arilo C_{6-10} , cicloalquilo C_{3-7} , heterociclo C_{3-10} aromático o no aromático, aril C_{6-10} -alquileno C_{1-6} , cicloalquil C_{3-7} -alquileno C_{1-6} y heterociclo C_{3-10} -alquileno C_{1-6} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por OH, oxo, alquilo C_{1-6} , fenilcicloalquilo C_{3-7} , heterociclo C_{3-7} y halógeno,

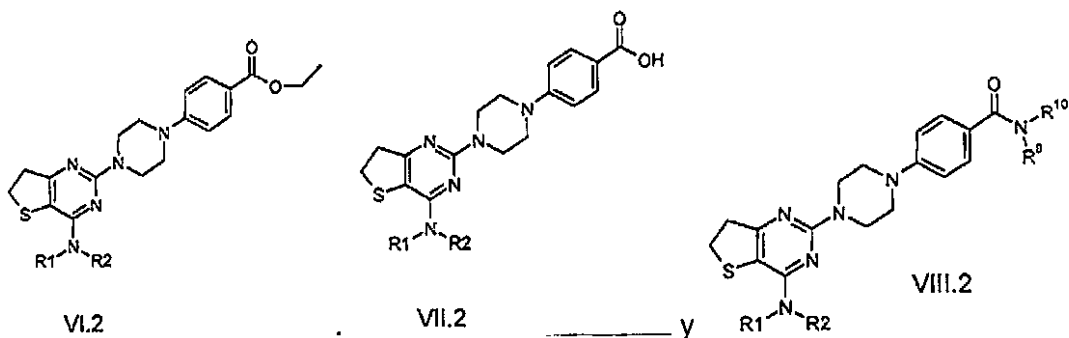
y en donde R^{13} está seleccionado del grupo compuesto por OH, halógeno, O-alquilo C_{1-6} , alquilo C_{1-6} , fenilo y cicloalquilo C_{3-7} y heterociclo C_{3-7} .

20. Productos intermediarios seleccionados del grupo compuesto por:

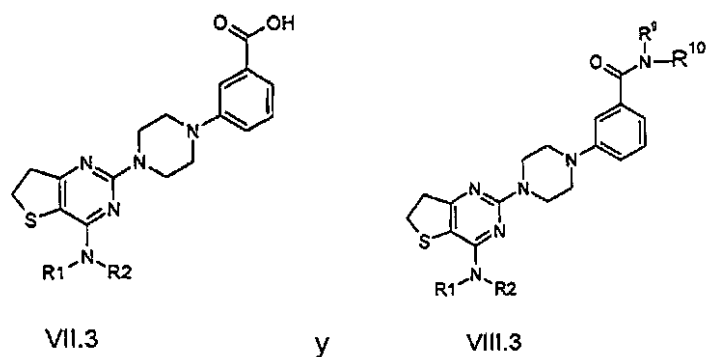
- compuestos de acuerdo con la fórmula I.1



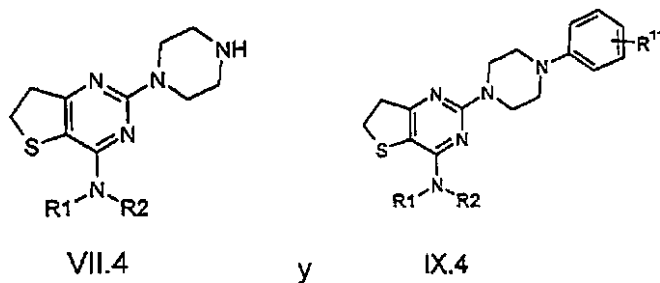
- compuestos de acuerdo con la fórmula VI.2, VII.2 y VIII.2



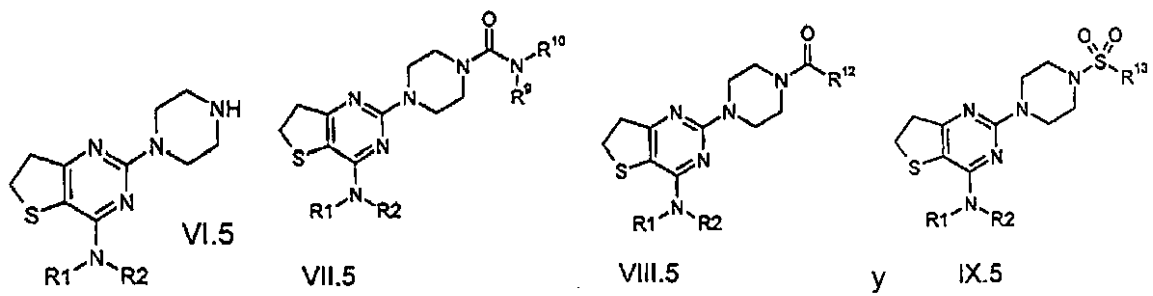
- compuestos de acuerdo con la fórmula VII.3 y VIII.3



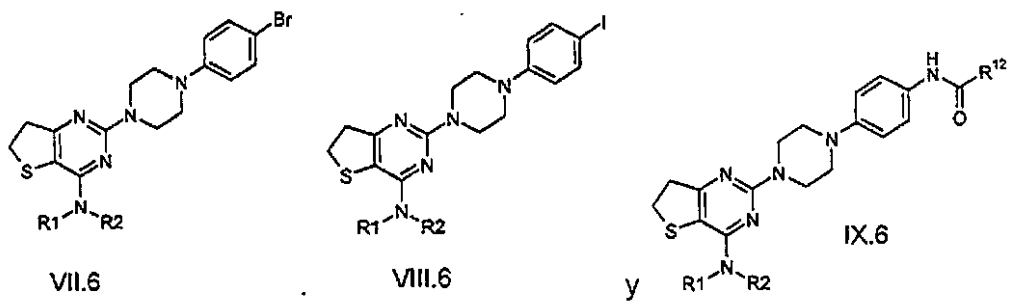
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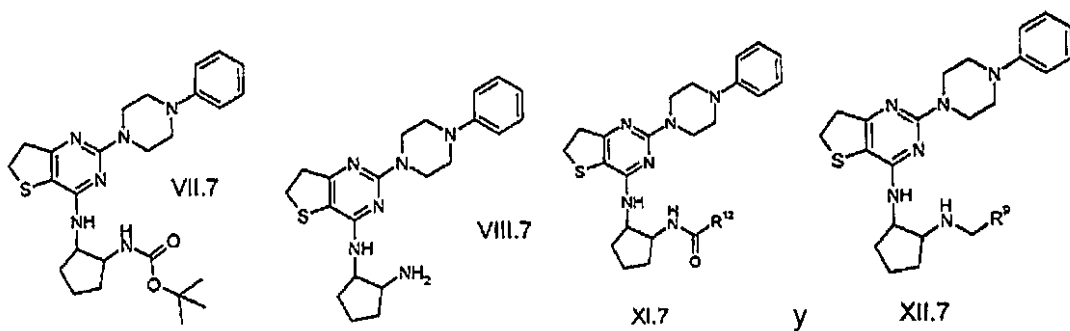
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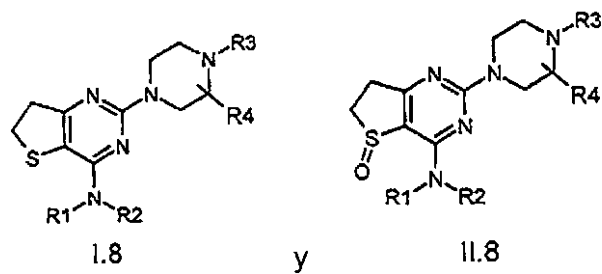
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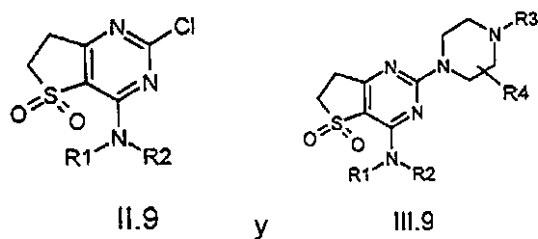
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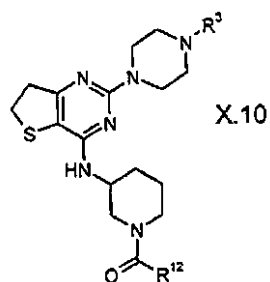
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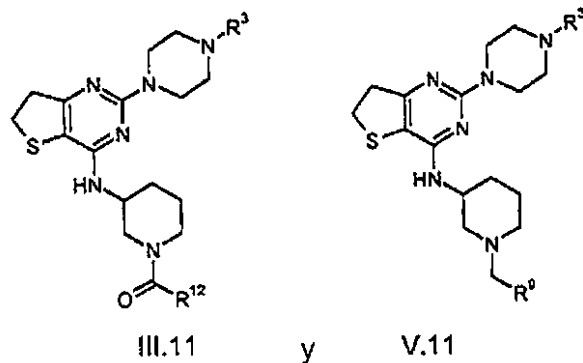
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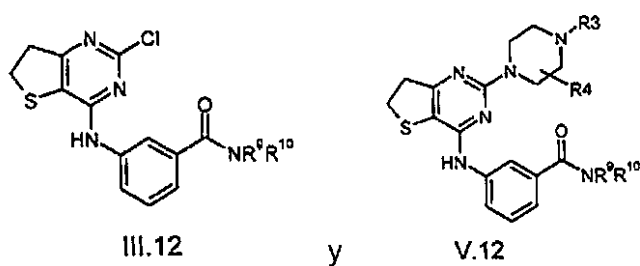
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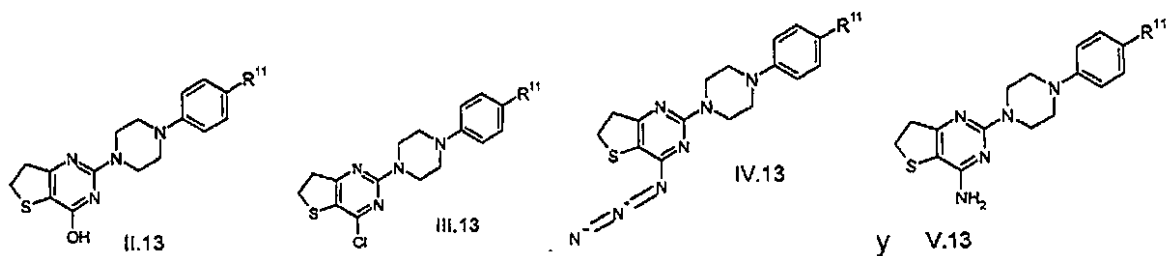
- compuestos de acuerdo con la fórmula III.11 y V.11



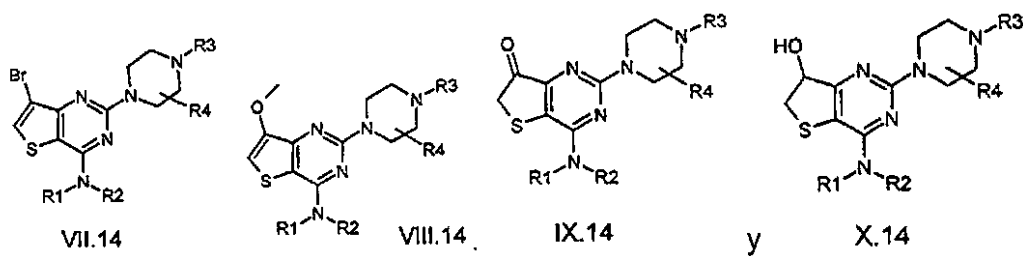
- compuestos de acuerdo con la fórmula III.12 y V.12



- compuestos de acuerdo con la fórmula II.13, III.13, IV.13 y V.13



- compuestos de acuerdo con la fórmula VII.14, VIII.14, IX.14 y X.14



en donde R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 y X tienen los significados definidos en las reivindicaciones 1 a 18,

y en donde R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 y X tienen los significados antes definidos y en donde R^9 , R^{10} , R^{11} , R^{12} son, en cada caso de modo independiente entre sí, un radical seleccionado del grupo compuesto por H, halógeno, alquilo C_{1-6} , haloalquilo C_{1-6} , arilo C_{6-10} , cicloalquilo C_{3-7} , heterociclo C_{3-10} aromático o no aromático, aril C_{6-10} -alquilenos C_{1-6} , cicloalquil C_{3-7} -alquilenos C_{1-6} y heterociclo C_{3-10} -alquilenos C_{1-6} , que eventualmente puede estar sustituido con uno o varios radicales seleccionados del grupo compuesto por OH, oxo, alquilo C_{1-6} , fenilcicloalquilo C_{3-7} , heterociclo C_{3-7} y halógeno,

y en donde R^{13} está seleccionado del grupo compuesto por OH, halógeno, O-alquilo C_{1-6} , alquilo C_{1-6} , fenilo y cicloalquilo C_{3-7} y heterociclo C_{3-7} .

21. Compuestos de acuerdo con una de las reivindicaciones 1 a 18 como medicamentos.

22. Formulaciones farmacéuticas caracterizadas por el contenido de uno o varios compuestos de la fórmula 1 de acuerdo con una de las reivindicaciones 1 a 18.

23. Formulación farmacéutica aplicable por inhalación, caracterizada por un contenido de un compuesto de fórmula 1 de acuerdo con una de las reivindicaciones 1 a 18.

Comparative Experiments:**1. Experimental Part:**

The Scintillation Proximity (SPA) Assay (GE Healthcare, Nr. TRKQ7090) is based on the detection of the different affinities of the cyclic 3'-5'-adenosine monophosphate (cAMP, low affinity) and the linear 5'-adenosine monophosphate (AMP, high affinity) to yttrium-silicate-scintillator beads. The cAMP-specific phosphodiesterase (PDE) PDE4B cleaves the 3'-phosphodiester bond of the tritium-labelled-[³H]cAMP to the [³H]5'-AMP. This [³H]5'-AMP associates with the scintillator beads because of their higher affinity and causes scintillations (light flashes) which can be measured in a Wallac Microbeta Scintillation Counter.

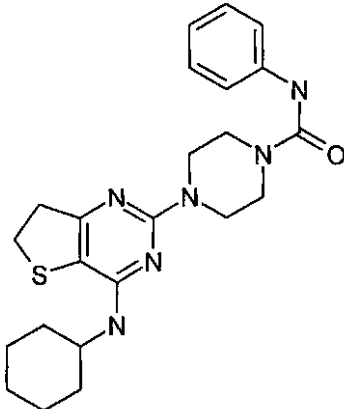
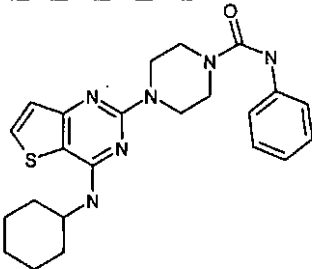
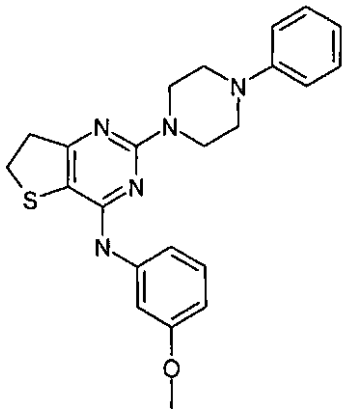
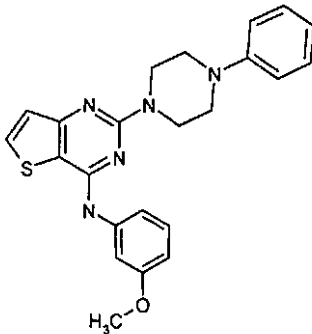
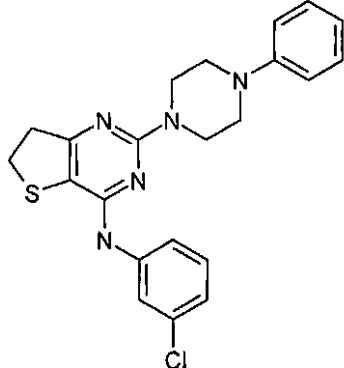
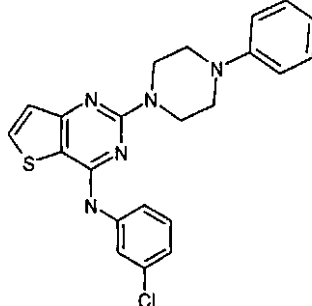
10 µl of a [³H]cAMP-Lösung (0.05 µCi in H₂O, 10 – 30 Ci/mmol) are added to 89 µl of a PDE4B-enzym-solution (0.15 ng) in assay buffer (50 mM Tris HCl pH 7.5; 8.3 mM MgCl₂; 1,7 mM EGTA; 0.25 mg / ml BSA) and this mixture is incubated at 30°C for one hour

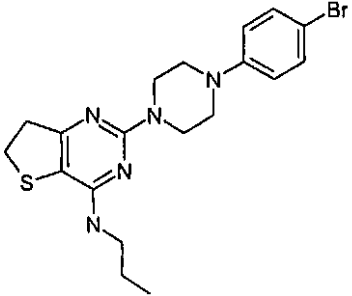
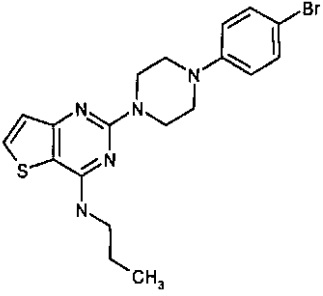
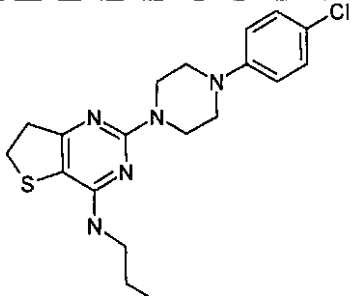
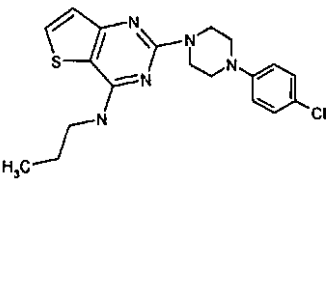
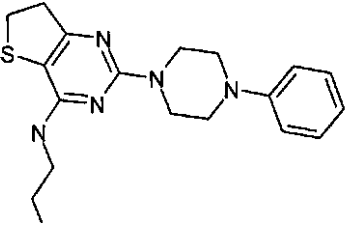
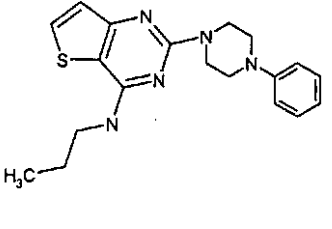
a) without the compound to be tested (in the presence of 1% DMSO) and
b) in the presence of the compound to be tested (DTP or corresponding TP) in a concentration of 125 µM, 25 µM, 5 µM, 1 µM, 200 nM, 40 nM, 8 nM, 1,6 nM, 0,32 nM, 0,064 nM, 0,0128 nM (dilution series in 5er-steps beginning from 125 µM until 0.0128 nM, in the presence of 1% DMSO).

After this incubation the reaction is stopped by the addition of 50 µl of bead-solution (500 mg beads / 35 ml H₂O, 18 mM zinc sulfate). In the following 45 minutes the beads have the opportunity to form a sediment. After that the scintillations are measured in the scintillation counter. If the tested compound is able to inhibit the enzymatic activity of the PDE4B-enzyme, less [³H]AMP depending on the concentration of the tested compound is produced and less scintillations are measurable. These results are expressed as IC₅₀-values (= compound concentration at which the enzyme activity is inhibited to a half maximal value).

2. Results:

Table 1:

structure of Dihydrothieno- pyrimidine (=DTP)	PDE4B IC ₅₀ - value of the DTP [μM]	structure of the corresponding thienopyrimidine (=TP)	PDE4B IC ₅₀ -value of the corres- ponding TP [μM]	ratio IC ₅₀ TP/ IC ₅₀ DTP
	0,0007		0,0079	11
	0,011		0,0590	5
	0,014		0,0700	5

structure of Dihydrothieno- pyrimidine (=DTP)	PDE4B IC ₅₀ - value of the DTP [μM]	structure of the corresponding thienopyrimidine (=TP)	PDE4B IC ₅₀ -value of the corres- ponding TP [μM]	ratio IC ₅₀ TP/ IC ₅₀ DTP
	0,02		0,2240	11
	0,0135		0,2443	18
	0,054		0,3420	6



PDE4 inhibition: a novel approach for the treatment of inflammatory bowel disease

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Inflammation is a hallmark of inflammatory bowel disease (IBD), and elevation of cAMP levels can inhibit the pro-inflammatory and tissue-destructive properties of leukocytes. Phosphodiesterase 4 (PDE4) is the predominant enzyme that metabolizes cAMP in inflammatory cells, and the anti-inflammatory and immunomodulatory potential of PDE4 inhibitors in human leukocytes, endothelium and epithelium is well documented. Although PDE4 inhibitors have been investigated as treatments for several inflammatory diseases, this has focused mainly on asthma and chronic obstructive disease (COPD). Historically, their clinical utility has been limited by nausea and emesis. However, the PDE4 inhibitors cilomilast and roflumilast have recently shown efficacy in asthma and COPD, with a reduced propensity to cause nausea and emesis. In this review, we summarize for the first time the evidence that PDE4 inhibitors might have therapeutic benefit in IBD, and discuss mechanisms of action beyond the inhibition of inflammatory cells.

The role of phosphodiesterase (PDE) enzymes is to regulate intracellular levels of the cyclic nucleotides cAMP and cGMP by catalyzing their breakdown to inactive 5'AMP and 5'GMP, respectively. There are 11 families of PDE enzymes (PDE1–PDE11), which are identified by substrate specificity, kinetic characteristics and amino acid sequence [1,2]. Type 4 PDE enzymes selectively hydrolyze cAMP ($K_m = 0.2\text{--}4.0\ \mu\text{M}$) and have a very low affinity for cGMP ($K_m > 1000\ \mu\text{M}$) [3]. Four gene products of PDE4 (PDE4A–PDE4D) exist, with multiple splice variants [4]. There is increasing evidence to indicate that the expression of PDE4 in specific compartments within cells tightly regulates cyclic nucleotide levels in the vicinity of effector proteins, thus implicating PDE4 in regulating cellular function. PDE4A, PDE4B and PDE4D are particularly abundant in many types of inflammatory and immune cells, including T cells and B cells, monocytes, macrophages, neutrophils and eosinophils. PDE4C is localized mainly to the testis, skeletal muscle, brain (with PDE4A, PDE4B and PDE4D), epithelium and human foetal lung [5,6].

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic, relapsing disease that can affect all parts of the gastrointestinal (GI) tract and is characterised by inflammation and tissue remodelling [7–9]. Key clinical features of the disease include abdominal pain, weight loss, diarrhoea and rectal bleeding [8]. IBD is thought to be a consequence of inappropriate and ongoing activation of the mucosal immune system that is driven by the presence of normal gut flora. It is likely that this abnormal response is facilitated by defects in the barrier function of the gut epithelium and the mucosal immune system.

The two main forms of idiopathic IBD, Crohn's disease (CD) and ulcerative colitis (UC), can be distinguished by the portion of GI tract that is affected and pathology. CD can affect any region of the GI tract whereas UC affects only the colon and rectum. The inflammatory process in UC is confined to the inner lining of the gut (mucosa and submucosa) and is characterised by crypt abscesses and ulcerations. Unlike UC, the inflammation in CD extends throughout the bowel wall (transmural) and is characterised by the presence of granulomas and fistulae [7]. Both T helper 1 (Th1) cells and Th2 cells induce chronic intestinal inflammation *in vivo*. Most Th1 models of chronic intestinal inflammation, such as trinitrobenzene sulfonic acid-induced colitis have transmural inflammation, as observed in patients with CD, whereas some of the Th2 models (e.g. T-cell receptor α -mutant mice) are characterised by a more superficial colonic inflammation that is more similar to UC [10].

Existing therapies are predominantly aminosalicylates and steroids. Although these provide therapeutic benefit and an improved quality of life for many patients, they are associated with a significant side-effect profile and a significant proportion of patients are either steroid resistant (~15–20%) or steroid dependent (~25–30%) [11]. In addition, new therapies are needed for IBD because: (i) existing therapies fail to induce and maintain remission in ~30% of patients; (ii) maintenance therapies have limited efficacy; and (iii) ultimately, a high proportion of patients (50% in CD and 20% in UC) require surgery [8,11,12]. The only therapy that has been introduced recently is the tumour necrosis factor α (TNF- α) antibody infliximab (Remicade®) for the treatment of steroid-refractory patients with CD. Although effective, 'biologicals' have disadvantages

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compared with small molecules, including restriction to non-oral routes of administration, immunogenicity and high cost [12].

Evidence that PDE4 inhibitors suppress inflammatory-cell activation

IBD is characterised by the recruitment of a range of inflammatory cell types to the gut, including T cells, macrophages and neutrophils. Furthermore, several cytokines, including interleukin 6 (IL-6), granulocyte-macrophage colony-stimulating factor, TNF- α and IL-8, and eicosanoids have been detected in the inflamed intestine [13]. In particular, TNF- α and interferon γ (IFN- γ), the latter of which can induce cytotoxicity [14], are implicated in the initiation and exacerbation of disease in humans and mouse models of IBD, and CD is characterised by hypersecretion of TNF- α and IFN- γ by T cells in the intestinal lamina propria [15]. TNF- α has many pro-inflammatory effects, including stimulating the expression of adhesion molecules on endothelial cells [16], elaboration of matrix metalloproteinases (MMPs) that contribute to tissue damage [17] and induction of major histocompatibility complex class II antigen expression on colonic epithelium [18]. TNF- α also stimulates the secretion of IL-8 [19] and monocyte chemoattractant protein 3 [20] from epithelial cells, both of which act as chemoattractants for other inflammatory cells. In addition, TNF- α can also stimulate T cells and natural killer (NK) cells to produce IFN- γ . Further support for a key role of TNF- α in IBD comes from the clinical efficacy of infliximab in steroid-refractory and fistulizing CD patients [21,22]. Similarly, antibodies to either TNF- α or IFN- γ prevent colitis and ameliorate inflammation in mouse models of IBD [23].

PDE4A, PDE4B and PDE4D are the predominant cAMP-hydrolyzing PDEs in most inflammatory cells and, in general, intracellular elevations in cAMP are associated with broad anti-inflammatory effects [1,6]. For example, PDE4 inhibitors are potent suppressors of many cytokines (Figure 1), including TNF- α release from macrophages [24],

monocytes [6] and T cells [25], which indicates that they could be effective in IBD but by a mechanism that is different from that of infliximab. In addition to inhibiting the activation of circulating inflammatory cells, PDE4 inhibitors inhibit the release of TNF- α and IFN- γ from T cells in the lamina propria [25]. In addition to their ability to suppress the release of pro-inflammatory mediators, evidence also indicates that PDE4 inhibitors stimulate the release of anti-inflammatory mediators such as IL-10 and prostaglandin E₂ [26,27], which are key anti-inflammatory regulators in the gut. PDE4 inhibitors also inhibit the increase in expression of cell adhesion molecules induced by IL-15 [28] and TNF- α [16], and interfere with platelet-activating factor (PAF)- and IL-8-directed T-cell chemotaxis [29]. The PDE4 inhibitor cilomilast also inhibits fibroblast-mediated collagen-gel degradation induced by TNF- α and neutrophil elastase by suppressing the release of MMP-1 and MMP-9 and reducing the conversion of MMP-1 to its active form [30]. Given that MMPs are associated with tissue destruction in IBD, this additional mechanism of action of PDE4 inhibitors might also contribute to their potential therapeutic value in IBD.

PDE4 inhibitors are effective in animal models

PDE4 inhibitors prevent (Table 1) and reverse (Table 2) GI inflammation and disease symptoms in several animal models. Although few studies have directly compared a PDE4 inhibitor with either a steroid or salicylates, where examined, the efficacy of a PDE4 inhibitor is equivalent to a steroid and superior to salicylates [31–34]. In preventative models, drug treatment is initiated either just before or concomitantly with disease induction, whereas in therapeutic models, drug treatment is instigated post-induction. A commonly used end-point in these studies is a 'clinical score' of colitis (Table 1). In addition, a reduction in colon length is a characteristic feature observed in many models of colitis and in human disease, and is considered to be a good surrogate marker of inflammation that can be confirmed histologically [35].

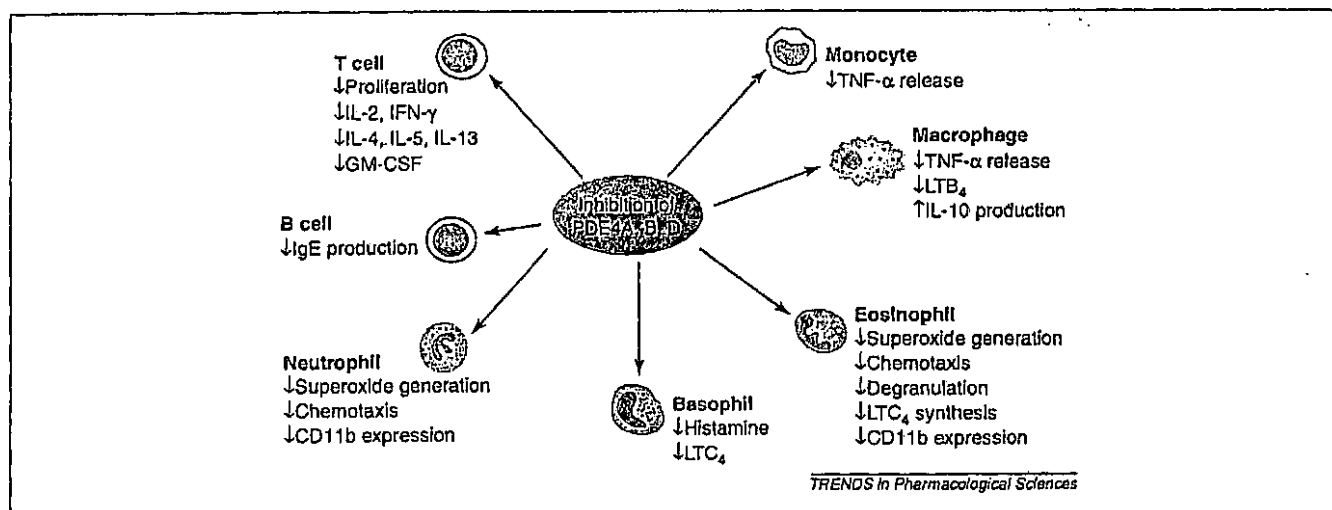


Figure 1. Potential anti-inflammatory and immunomodulatory effects of phosphodiesterase 4 (PDE4) inhibitors. PDE4 inhibitors are potent suppressors of a wide range of cytokines from several inflammatory cell types [6,24,25]. In addition to their ability to suppress the release of pro-inflammatory mediators, PDE4 inhibitors can stimulate the release of interleukin 10 (IL-10) from monocytes [26]. This cytokine is a key anti-inflammatory regulator in the gut. Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN- γ , interferon γ ; LTB₄, leukotriene B₄; TNF- α , tumour necrosis factor α .

Table 1. PDE4 inhibitors prevent colitis in animal models^a

Compound	Model	End-points ^b				Refs
		Improvement in clinical score	Reversed reduction in colon length	Reduction in mediator levels	Improved histological score	
Rolipram (10 mg kg ⁻¹ i.p. o.d. for 11 days)	DSS colitis (mouse)	Yes	Yes	TNF- α (colonic)	Yes	[35]
Mesopram (10 mg kg ⁻¹ p.o. o.d. for 11 days)	DSS colitis (mouse)	Yes	Yes	NSE on TNF- α and IFN- γ	Yes	[31]
Rolipram (5 mg kg ⁻¹ i.p. b.i.d. for 5 days)	DSS colitis (mouse)	NI	Yes	MPO (colonic)	Yes	[38]
Rolipram (1 mg kg ⁻¹ p.o., single dose)	Indomethacin (rat)	Yes	Yes	NI	NI	c

^aAbbreviations: b.i.d., twice daily; DSS, dextran sodium sulfate; IFN- γ , interferon γ ; i.p., intraperitoneal; MPO, myeloperoxidase; NI, not investigated; NSE, no significant effect; o.d., once daily; p.o., oral; PDE4, phosphodiesterase 4; TNF- α , tumour necrosis factor α .

^bClinical score is a symptomatic grading of elements related to human disease that often includes body weight loss, stool consistency and faecal blood. Histological score is a grading of colitis severity that is normally scored according to the extent and severity of inflammatory changes in mucosal architecture and morphometry.

^cK.H. Banner and M.A. Trevethick, unpublished.

A widely used animal model of IBD is the dextran sodium sulfate (DSS) model of colitis, and drugs that are used clinically for IBD such as salicylates and anti-TNF- α antibodies demonstrate good efficacy in this model [36,37]. DSS colitis is characterised histopathologically by mucosal infiltration of inflammatory cells, focal crypt damage, epithelial injury and ulceration. The pathology is similar

to CD in terms of the transmural extent of the inflammation and aphthous erosions, but no fissures and granulomas are seen. The pathogenesis of this model of colitis is believed to be caused by a direct toxic effect of DSS on the epithelium followed by cytokine production from macrophages as a consequence of phagocytosis of DSS. The effect of mesopram, a selective PDE4 inhibitor that is

Table 2. PDE4 inhibitors reverse established colitis in animal models^a

Compound and dosing schedule post-induction of colitis ^b	Model	End-points ^c				Refs
		Improvement in clinical score	Reversed reduction in colon length	Reduction in levels of cytokines or cell number	Other	
Rolipram (3.75–7.5 mg kg ⁻¹ p.o. b.i.d.) Arofyline (15–30 mg kg ⁻¹ p.o. b.i.d.) Prednisolone (7.5 mg kg ⁻¹ i.m. o.d. on days 8–12)	DSS (rat)	All decreased bleeding	NI	LTB ₄ (colonic) reduced by all treatments MPO (colonic) reduced only by arofyline	No change in histological score	[33]
Rolipram (10 mg kg ⁻¹ i.p. o.d. on days 8–15)	DSS (mouse)	Yes	Yes	NI	NSE on histological score	[35]
Mesopram (10 mg kg ⁻¹ i.p. o.d. on days 8–14)	DSS (mouse)	Yes	Yes	IFN- γ (colonic)	NSE on histological score	[31]
OPC6535 (1 mg kg ⁻¹ p.o. o.d.) SAP (50 mg kg ⁻¹ p.o. b.i.d. on days 15–43)	DSS (rat)	Both agents reduced incidence of diarrhoea and bloody stools	NI	NI	Both agents reduced the area of intestinal inflammation	[58]
Rolipram (10 mg kg ⁻¹ p.o. o.d.) Methylprednisolone (5 mg kg ⁻¹ i.m. o.d. on days 7–14)	TNBS (rat)	NI	NI	Colonic PGE ₂ , LTB ₄ , MPO, TNF- α and TGF-1 β reduced by both treatments	Only rolipram reduced tissue collagen	[34]
OPC6535 (1 mg kg ⁻¹ p.o. o.d.) SAP (50 mg kg ⁻¹ p.o. b.i.d. on days 2–8)	TNBS (rat)	Both agents reduced incidence of diarrhoea	NI	Only OPC6535 reduced neutrophils	Only OPC6535 reduced intestinal inflammation	[32]

^aAbbreviations: b.i.d., twice daily; DSS, dextran sodium sulphate; IFN- γ , interferon γ ; i.m., intramuscular; i.p., intraperitoneal; LTB₄, leukotriene B₄; MPO, myeloperoxidase; NI, not investigated; NSE, no significant effect; o.d., once daily; PDE4, phosphodiesterase 4; PGE₂, prostaglandin E₂; p.o., oral; SAP, salazosulapyridine; TGF-1 β , transforming growth factor 1 β ; TNBS, trinitrobenzene sulfonic acid; TNF- α , tumour necrosis factor α .

^bCompounds were administered separately.

^cClinical score is a symptomatic grading of elements related to human disease that often includes body weight loss, stool consistency and faecal blood. Histological score is a grading of colitis severity that is normally scored according to the extent and severity of inflammatory changes in mucosal architecture and morphometry.

currently in clinical development as an anti-inflammatory treatment for multiple sclerosis, has been evaluated in the DSS model [31]. In this study, mesopram (10 mg kg^{-1}) was an effective treatment for established DSS-induced colitis and also prevented colitis induction in a dose-dependent manner ($2\text{--}50 \text{ mg kg}^{-1}$). Specifically, in both the preventative and the therapeutic models, mesopram significantly reduced the clinical score and decreased colon shortening. In addition, in the preventative model, a reduction in histological grading of colitis severity was observed and, in the therapeutic model, mesopram significantly reduced *ex vivo* colonic production of IFN- γ . Using the same model of colitis, treatment with rolipram (10 mg kg^{-1}) in both preventative and therapeutic models improved the clinical score and either prevented or reversed the reduction in colon length, respectively [35]. Rolipram improved the histological score in the preventative model but there was only a trend towards improvement in the therapeutic model. In addition, rolipram reduced colonic TNF- α in the preventative setting. A separate study has also demonstrated that rolipram (5 mg kg^{-1} twice daily) administered concomitantly with DSS for 5 days significantly reduces the severity of colonic histopathology and myeloperoxidase levels [38].

The indomethacin-induced ulceration model in rodents is another model of gut ulceration with similarities to human CD [39]. This model is characterised by a transmural inflammation with discontinuous focal ulceration. We have shown that a single oral dose of rolipram ($1\text{--}10 \text{ mg kg}^{-1}$) 1 h before administering indomethacin subcutaneously can prevent ulceration and reduction in colon length, improve the histological grading of colitis, and reduce disease symptoms in a dose-dependent manner (K.H. Banner and M.A. Treveathick, unpublished).

Given that tissue remodelling is an important feature of IBD, it is of note that the PDE4 inhibitor roflumilast significantly inhibits airway remodelling in a murine model of chronic asthma, as shown by a reduction in sub-epithelial collagenization and thickening of airway epithelium [40]. If these effects translate to the gut epithelium, it might contribute to a therapeutic benefit in IBD.

In summary, PDE4 inhibitors can suppress the release of inflammatory mediators from several inflammatory cell types implicated in IBD and prevent and ameliorate gut inflammation in animal models of IBD. This indicates that PDE4 inhibitors might represent novel treatments for IBD.

Clinical experience with PDE4 inhibitors

Several PDE4 inhibitors are being evaluated in the clinic and have shown promising efficacy for the treatment of asthma and COPD [6]. The most advanced of these is cilomilast, which has been developed by GlaxoSmithKline (<http://www.gsk.com/>) and received an approvable letter for the treatment of COPD from the Food and Drug Administration (FDA) in October 2003 (<http://www.researchtriangle.org/news/nr/oct272003a.html>). Recent clinical studies demonstrate that treatment of COPD patients with cilomilast (15 mg twice daily for 10 weeks) significantly reduced the numbers of CD8 $^{+}$ T cells, macrophages and neutrophils in bronchial biopsies [41]. This is the first demonstration that inhibition of PDE4 reduces

the number of inflammatory cells in human airway tissue. Selective and potent inhibitors of PDE4 have not yet been evaluated in clinical trials for the treatment of IBD. Pentoxifylline (400 mg , 4 times daily), a weak, nonselective PDE inhibitor has been evaluated, but was ineffective in a clinical trial of patients with corticosteroid-dependent, chronic, active CD [42]. Given that pentoxifylline is also a weak inhibitor of TNF- α release from human peripheral blood mononuclear cells (IC_{50} for inhibition of TNF- α synthesis by these cells is $70 \mu\text{M}$) [24] and TNF- α was not measured in this study, it is not known whether this dose of pentoxifylline is sufficient to reduce TNF- α in the inflamed gut.

Another relatively weak PDE4 inhibitor, CC1088 (see Chemical names; IC_{50} for PDE4 inhibition = $15 \mu\text{M}$; IC_{50} for TNF- α inhibition = $21 \mu\text{M}$) completed a Phase I–Phase II, double-blind, placebo-controlled trial to evaluate safety and efficacy in patients with moderate to severe CD [43; <http://ir.celgene.com/phoenix.zhtml?c=111960&p=irol-newsArticle&ID=368043>]. Six out of 11 patients who received either 800 mg or 1200 mg of CC1088 responded positively, as defined by a decrease of ≥ 70 points in the CD Activity Index at week 12, compared with three of the 7 patients on placebo who were evaluated. This trend is notable because the patients in the placebo arm had significantly less disease activity on entry to the trial.

Lastly, an Otsuka (<http://www.otsuka.com/>) compound, OPC6535, which is reported to be a more potent inhibitor of PDE4 (IC_{50} = $0.07 \mu\text{M}$ for inhibition of superoxide release from human neutrophils) than pentoxifylline and CC1088, entered a randomised, double-blind, placebo-controlled, parallel arm, safety and efficacy trial investigating once-daily oral treatment in active UC. OPC6535 showed promise in the treatment of UC, particularly in patients with greater disease severity, with or without concurrent 5-aminosalicylate use (S.B. Hanauer, unpublished; <http://www.ddw.org/media/newsReleases/IBDTherapies.pdf>). We are unaware of information on the PDE selectivity of the two 'novel' PDE4 inhibitors OPC6535 and CC1088.

Is the anti-inflammatory action of PDE4 inhibitors the only mechanism that explains their ability to inhibit ulceration?

The effects of PDE4 inhibitors on both the inflammatory and the immunomodulatory function of leukocytes *in vitro* and *in vivo* are likely to explain their efficacy in clinical and preclinical models of inflammation. Increases in cAMP also lead to the relaxation of smooth muscle.

The role of the vasculature and disturbances of blood flow are thought to be important pathogenic mechanisms in IBD. Vascular changes do occur in the mucosa and submucosa in both CD and UC [44], although it was assumed that these changes were secondary to the inflammatory processes occurring. Angiographic studies in CD and UC have demonstrated significant abnormalities in the vasa recta that correlate with disease extent and severity. In particular, advanced IBD is associated with decreased vascular density and diminished blood flow. Measuring intestinal blood flow by several techniques

shows that there is an increase in vascular perfusion in early stages of disease, whereas reduced blood flow is seen as the disease progresses [44]. Fresh impetus for the 'vascular hypothesis' was provided by Wakefield and colleagues, who suggested that vascular injury and arteritis were early events that occurred in parts of the gut with an apparently normal mucosa [45]. Arterial occlusion and infarction in multiple sites follows and mucosal damage occurs as a consequence of changes in blood flow. Further exacerbation of the disease process occurs as a consequence of inflammatory cell infiltration. Subsequent studies by other investigators support this hypothesis [46]. Preclinical models of IBD show that decreases in blood flow occur early following administration of ulcerogenic stimuli [47], thus predisposing to mucosal ulceration and subsequent inflammation. Abnormal patterns of contractility have been observed in human IBD [48], and spasm of the colonic muscularis mucosae was believed to be important in the pathogenesis of UC, which is treatable with smooth-muscle relaxants [49]. Preclinical models show that hyper-contractility of the gut, which is induced by agents such as indomethacin, can lead to mucosal ulceration by decreasing the mucosal blood flow [50].

Many mechanisms have the potential to alter blood flow to the intestine (Figure 2). In addition to leukocytes, PDE4 also occurs in animal and human gut [51,52], and inhibition of PDE4 *in vitro* causes relaxation and inhibition of spontaneous contractions of gut smooth muscle. Therefore, inhibition of PDE4 in the gut is expected to decrease contractility of gut smooth muscle, thus lessening compression of the vasculature and helping to maintain mucosal blood flow. Such a concept is postulated to be the major mechanism whereby β_2 -adrenoceptor agonists inhibit indomethacin-induced ulceration of the small intestine in rats [53].

PDE4 inhibitors as a treatment for IBD

Currently, nausea and emesis appear to be the major side-effects that might limit the therapeutic index of non-PDE4 isoform-selective inhibitors such as cilomilast and roflumilast [6]. In animal studies, vasculitis, a pathological inflammation of the blood vessel wall and surrounding tissue, has also been noted [54]. Whether vasculitis is an issue clinically for PDE4 inhibitors remains to be determined [6].

It is not known which isoform of PDE4 is responsible for inducing emesis and vasculitis, but PDE4D is claimed to be associated with emesis in animal models [55]. In addition, there is evidence to indicate that the induction of emesis involves both CNS and gut components [56]. Thus, the development of orally administered PDE4 inhibitors faces challenges but the emerging clinical properties of roflumilast and cilomilast should clarify these concerns. Unless nausea and emesis are mediated by a specific PDE4 isoform that is different from the isoform that controls inflammation and blood flow, emesis is likely to reduce the potential of these agents in treating IBD. Although the mechanism is unknown, if it is related to the circulating levels of PDE4 inhibitors, then there are potential strategies to overcome this side-effect in gut diseases. One strategy is to target their action to the gut *per se* by developing oral inhibitors of PDE4 that are either not absorbed into the portal circulation or that undergo extensive first-pass metabolism. This concept is not new. For example, the corticosteroid budesonide is indicated for the treatment of IBD. Budesonide undergoes significant first-pass metabolism in the liver following oral administration and is associated with fewer side-effects than conventional, orally bioavailable steroids such as prednisolone. However, further long-term analysis is required, to determine the efficacy of this treatment in IBD [57].

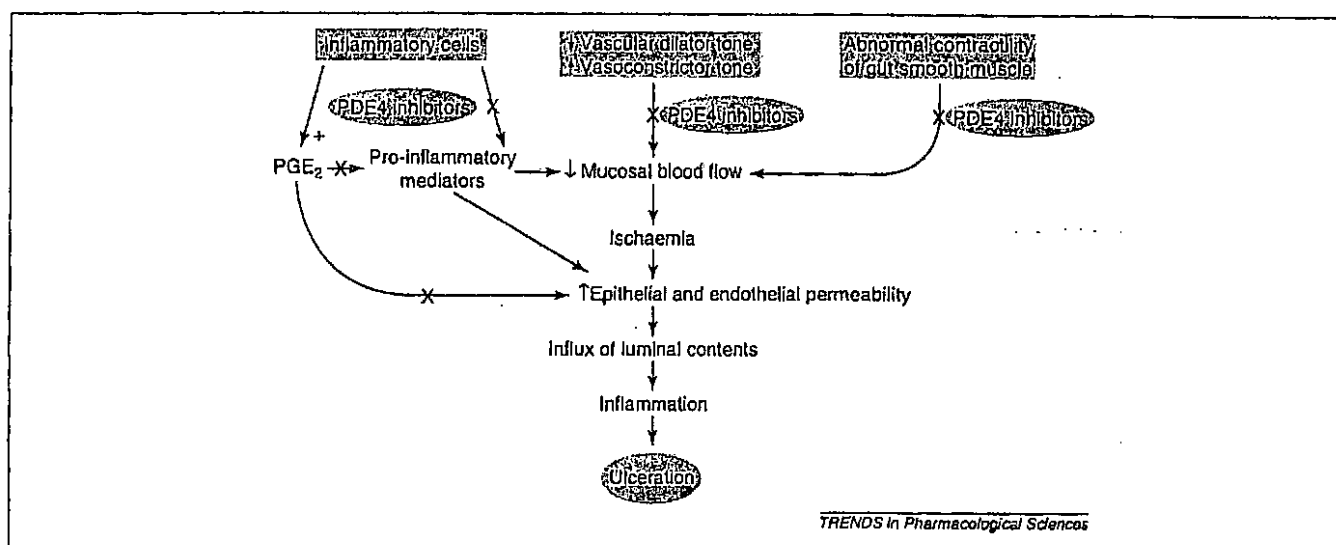


Figure 2. Abnormalities in inflammatory bowel disease (IBD) and the potential beneficial effects of phosphodiesterase 4 (PDE4) inhibitors. Tissue ulceration can be caused by several potential mechanisms. Inflammation *per se* can lead to tissue destruction and alter the contractility of vascular and gut smooth muscle, which act together to decrease mucosal blood flow and, ultimately, to the loss of gut luminal permeability. This leads to an influx of luminal contents and provides a positive drive to increase inflammation, decrease mucosal blood flow and enhance tissue destruction. Many mechanisms have the potential to alter blood flow to the intestine. These include: (i) blockage of mucosal blood vessels by microthrombi [45]; (ii) reduction of vasodilator responses of blood vessels that can be induced by pro-inflammatory mediators such as reactive oxygen species [44]; (iii) constriction of vascular and gut smooth muscle induced by pro-inflammatory mediators [44]; and (iv) compression of blood vessels by abnormal contractility of gut smooth muscle induced, for example, by inflammatory mediators. Apart from (i) evidence indicates that PDE4 inhibitors inhibit the mechanisms that can alter intestinal blood flow. Elevations of cAMP levels potentially inhibit the release of pro-inflammatory mediators, relax gut and vascular smooth muscle and, thus, provide a broad-spectrum action to inhibit tissue destruction. Abbreviation: PGE₂, prostaglandin E₂.

Chemical names

CC1088: 3-[3-(cyclopentylloxy)-4-methoxyphenyl]-3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propanamide
 OPC6535: 6-[2-(3,4-diethoxyphenyl)thiazol-4-yl]-pyridine-2-carboxylic acid

Concluding remarks

The profile of PDE4 inhibitors *in vitro* and *in vivo* indicates that these agents might be novel, powerful anti-inflammatory agents for IBD because they reduce the pro-inflammatory properties of leukocytes and maintain mucosal blood flow. This profile also has the advantage of a spectrum of biological activities that is not found in current medications. Although there are potential concerns associated with orally administered PDE4 inhibitors, the profiles of cilomilast and roflumilast should do much to address these. In addition, if PDE4-mediated side-effects are associated with systemic exposure, then compounds that are either not absorbed into the portal circulation or that undergo extensive first-pass metabolism might have an improved safety profile.

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The next generation of PDE4 inhibitors

Zheng Huang*, Yves Ducharme, Dwight Macdonald and Annette Robichaud

A number of highly potent PDE4 inhibitors are being developed for the treatment of asthma, chronic obstructive pulmonary disease, rheumatoid arthritis, multiple sclerosis and Crohn's disease.

Cilomilast (Aristo™, SB 207499, SmithKline Beecham), the most advanced member of the class in Phase III clinical trials, was reported to have a limited therapeutic window. Other inhibitors with improved profiles in preclinical models are entering into (or are in) clinical trials. The recent developments in understanding PDE4 catalysis, inhibitor binding and their emetic response should facilitate the design of the next generation of PDE4 inhibitors.

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Current Opinion in Chemical Biology 2001, 5:432–438

1367-5931/01/\$ — see front matter

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Abbreviations

CNS central nervous system
COPD chronic obstructive pulmonary disease
LPS lipopolysaccharide
PDE phosphodiesterase

Introduction

Of the 11 families of phosphodiesterase (PDE) responsible for the hydrolysis of cAMP and cGMP [1,2], the cAMP-specific PDE4s are encoded by four genes (A–D) [3**]. PDE4s are particularly abundant in inflammatory and immune cells and in airway smooth muscles, where inhibition of PDE4 blocks cell trafficking, cell proliferation and attenuates the production of inflammatory mediators, cytokines and reactive oxygen species [4,5**]. PDE4 inhibitors are generally anti-inflammatory and bronchodilatory in animal models [4,5**,6]. A number of them are being evaluated in the clinic and they have shown promising clinical efficacy for asthma [7,8*], chronic obstructive pulmonary disease (COPD) [6,9**] and atopic dermatitis [10]. However, PDE4 inhibitors also produce the characteristic dose-limiting side effects of nausea and emesis that have hampered their clinical development [11–13]. Cilomilast (1, Figure 1), which recently completed Phase III clinical trials, also suffered from a limited therapeutic index because of nausea and emesis [9**]. To complement recent reviews on PDE4 biology [3**,14,15], PDE4 inhibitors [16**,17*] and their therapeutic potential [4,5**,18,19*], this article focuses on recent developments (2000–2001) in understanding PDE4 catalysis, inhibitor binding and their emetic response. In addition, it highlights several PDE4 inhibitors that were reported to have improved pre-clinical profiles during this period.

Complexity and specificity of PDE4-mediated signalling

Each of the four PDE4 genes encodes a number of splice variants that share identical catalytic and carboxy-terminal

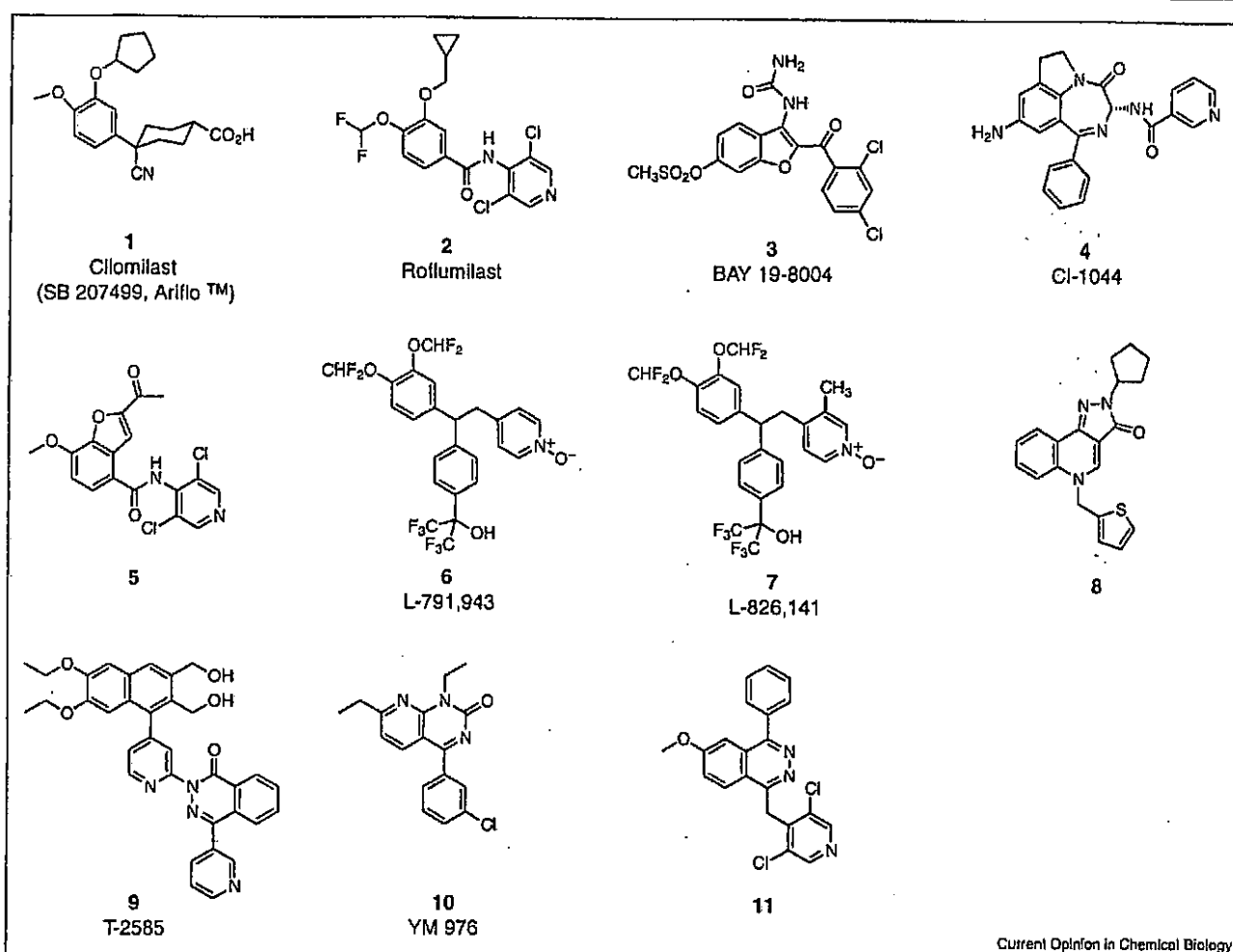
domains. The total number of PDE4 gene products identified exceeds 17 and their tissue-specific expression is tightly regulated in response to hormones and other stimuli [3**,14,20]. Their upstream conserved regions (UCRs) and divergent amino termini contain signals for localisation, protein–protein interaction and activity regulation, thereby allowing the fine-tuning of cAMP signalling to discrete subcellular compartments and specific pathways (see also Update). For example, PDE4 expression is regulated by various stimuli including histamine [21], TNF α [20], electroconvulsive shock [22], presynaptic noradrenergic activity [23], antidepressant treatment [24] and the activation of cAMP-dependent and mitogen-activated protein kinase signalling pathways [25–27]. In addition, PDE4 activities are modulated by PKA- and Erk2-mediated serine phosphorylations [28–32] and phosphatidic acid binding [33]. The detection of 13 out of 14 cAMP- and cGMP-PDE genes evaluated in human cavernous smooth muscle [34] and the absence of the muscarinic/cholinergic airway responses in PDE4D $^{-/-}$ mice [35**] illustrate the complexity and specificity of the cyclic nucleotide-mediated signalling and the nonredundant role for each protein.

PDE4 catalytic machinery

PDE4 enzymes are divalent cation-dependent hydrolases [36,37]. The atomic structure of PDE4B [38**] and a detailed binding study [39**] have provided new insights into its catalytic machinery, substrate specificity and inhibitor binding and have clarified the molecular origin of its two conformational states. The active site is formed by hydrophobic and negatively charged amino acids residing on several α helices. Of the 21 residues absolutely conserved across all 11 families of PDEs, 8 are near and 12 are within this pocket. Two divalent cations, in a binuclear motif, are bound to residues conserved among all PDEs. One of them (M_1) coordinates to His238, His274, Asp275 and Asp392, whereas the other (M_2) is loosely bound to Asp275 and M_1 via a hydroxyl in PDE4B. The metal ions could provide an activated hydroxyl nucleophile to hydrolyse the phosphodiester bond. The metal ions are also responsible for inducing high affinity and productive cAMP binding, probably by chelating the phosphate oxygens of cAMP, which leads to an increased phosphate electrophilicity. In the absence of the cofactor ions, cAMP binds weakly and non-productively to the active site. The cAMP specificity in PDE4s is mainly conferred by an aspartic acid, which is replaced by an asparagine in cGMP-PDEs [40] (see also Update).

The current model of PDE4 catalysis supports the hypothesis that altering the cofactor binding affinity of PDE4 might be involved in enzyme regulation [28,39**]. The binuclear ion centre is located at the interface of three subdomains [38**]. It is tempting to speculate that the

Figure 1



PDE4 inhibitors discussed in this review.

enzyme's metal-ion-binding affinity may be sensitive to perturbations within these subdomains. This hypothesis seems to be supported by the increased Mg^{2+} binding upon Ser54 phosphorylation [28,41] and the variable Mg^{2+} binding affinities among PDE4 mutants [42].

Molecular origins of the two PDE4 conformers

The reversible metal ion cofactor binding results in the presence of two coexisting PDE4 conformers (apoenzyme and holoenzyme) that bind inhibitors differentially [39**]. Because inhibitor binding to either conformer leads to inhibition of catalysis, this causes the inhibitor potency to partition between its apoenzyme and holoenzyme binding affinity. The high affinity rolipram-binding site of PDE4, which was previously thought to be distinct from its catalytic site [43,44], is in fact the holoenzyme active site. In contrast, rolipram binds weakly to the apoenzyme [39**]. The metal-ion-dependent and independent components of inhibitor binding to the active site provide the opportunity of using ion-chelating motifs within inhibitors to improve their potency.

Emesis response of PDE4 inhibitors

Nausea and emesis remain the major obstacles in developing PDE4 inhibitors [9**,11,12,45,46]. Because all PDE4 inhibitors are emetic to a certain degree, it suggests that cAMP elevation in emetic centres is coupled with their emetic response. However, whether emesis is mediated via a specific PDE4 subtype remains to be established. Several PDE4 subtypes have been identified in areas of the brain that have been implicated with the emetic response [22,23,47,48]. The recent identification of an emetic, efficacious and competitive PDE4 inhibitor capable of covalently labelling its biological targets upon photoactivation could prove to be a useful tool in the identification of both the emesis and the beneficial targets of PDE4 inhibition [49*].

PDE4 inhibitors could activate the emetic reflex via a peripheral and a central locus of action and multiple mediators may be involved [12,50**]. The emetic action of some inhibitors may be mediated through a potentiation of serotonergic neurotransmission in the vagal nerve afferent

The Tanabe group [66] has selected T-2585 (9), a 0.13 nM PDE4 inhibitor for further evaluation as an anti-asthmatic agent. In the guinea pig, it blocks antigen-induced bronchoconstriction ($ED_{50} = 63 \mu\text{g/kg i.v.}$) and histamine-induced bronchoconstriction ($ED_{50} = 33 \mu\text{g/kg i.d.}$). T-2585 is not emetic in ferrets and dogs at doses up to 10 mg/kg p.o. and 0.3 mg/kg i.v., respectively.

The Yamanouchi group [67,68] reported the efficacy and emesis data in ferrets for YM976 (10), a 2.2 nM inhibitor of the human leukocyte PDE4 (117 nM for cilomilast). In carrageenan-induced pleurisy in ferrets, YM976 inhibited the infiltration of leukocytes by 52% at 10 mg/kg p.o. (cilomilast was not active at 3 mg/kg p.o.). YM976 was not emetic in ferrets at 10 mg/kg p.o., whereas cilomilast was emetic at the same dose. YM976 also inhibited the antigen-induced lung eosinophilia in ferrets with an ED_{50} of 1.2 mg/kg p.o.

The Zambon group [69,70] has described several series of phthalazine derivatives. Compound 11 is slightly more potent than cilomilast (PDE4: $IC_{50} = 37 \text{ nM}$ and 73 nM , respectively) but it is not emetic in dogs at 30 mg/kg, i.v., whereas cilomilast is emetic at 10 mg/kg i.v.

Little is known about the biochemical factors that influence the therapeutic index of PDE4 inhibitors. For some of the previously described compounds, preclinical data in various animal models indicate that the emetic threshold is significantly higher than the efficacious dose. Selective targeting of a subpopulation of PDE4 proteins, either via a preferential drug distribution or subtype-selectivity, could be responsible for their improved profile. Some compounds may have subtype-selectivity that has yet to be reported or discovered.

The nonselective inhibition of multiple PDE4s may provide an explanation for the complex physiological responses resulting from their inhibition. One approach to reduce the side-effect profile of PDE4 inhibitors would be to develop subtype-selective inhibitors, taking advantage of their functional and distribution difference. Despite the similarity among the four PDE4 active sites [38**], naphthyridine-based PDE4D-selective [71] and catechol-based PDE4A/4B preferred inhibitors [72] have been reported. The atomic structure of the PDE4 active site will undoubtedly promote the further optimisation of these selective leads with the possibility of highly subtype-selective inhibitors to emerge in the near future.

Therapeutic potentials of PDE4 inhibitors

PDE4 inhibitors are currently in clinical trials for the treatment of asthma, COPD, atopic dermatitis, rheumatoid arthritis, multiple sclerosis and Crohn's disease. The rationale behind these therapeutic areas have been discussed in detail in several recent reviews [5,17*,18,73–75]. Here we highlight new evidence to support their potential utilities in other indications.

Cellular studies of PDE4 inhibitors indicate that their potency is positively coupled to the rate of cAMP turnover, a phenomenon that has been termed 'flux-mediated sensitivity' by Houslay and Milligan [15]. If the phenomenon is transferred *in vivo*, it would support the hypothesis that PDE4 inhibitors are more effective at targeting disorders and pathways undergoing higher cAMP turnover. In this situation, PDE4 inhibitors may have an increased therapeutic index. The potential synergism between the activation states of adenylate cyclase and the potency of a PDE4 inhibitor has led to the proposals that co-administering a PDE4 inhibitor with a β -agonist or leukotriene antagonist might provide additional efficacy and increase their therapeutic index in asthma [76–78,P2]. Rolipram attenuated syncytial virus-induced airway hyper-responsiveness and lung eosinophilia, suggesting PDE4 inhibitors may have potential in treating exacerbations of asthma triggered by viral infection [79]. PDE4 is the dominant cAMP-hydrolysing enzyme in 41 out of 60 human tumour cell-lines evaluated [80,81], supporting the potential of PDE4 inhibitors as antitumor agents. Despite the presence of multiple PDEs in chronic lymphocytic leukaemia cells (CLL), PDE4 inhibition results in the selective induction of apoptosis in CLL, while the normal T cells are unaffected [82,83]. HIV-1 infection of CD4⁺ T cells also requires the expression of PDE4 [84] and Rolipram inhibits HIV-1 viral replication [85]. PDE4 inhibitors protect the renal and mesenteric circulation and improve the survival rate in an endotoxin-induced model of multiple organ dysfunction syndrome [86,87]. In addition, PDE4 inhibition reverses memory deficits associated with NMDA receptor antagonism, suggesting a potentially novel mechanism for cognitive enhancement [88,90].

Conclusion

The therapeutic potential of PDE4 inhibitors in the clinic has thus far been hampered by their dose-limiting side effects of nausea and emesis. The recent advances in understanding the molecular interactions between PDE4 and its inhibitors, the specific functions of PDE4 isozymes and the emetic response of PDE4 inhibitors should open new research avenues and modify our approaches to identifying the next generation of PDE4 inhibitors. The atomic structure of PDE4 and the ability to dissect the cofactor-dependent and independent binding components of inhibitors should facilitate the practice of structure-based design. PDE4 inhibitors with improved preclinical profiles are currently being evaluated and may provide exciting opportunities to combat several human diseases in the near future.

Update

The substrate and inhibitor specificities of PDE4 have recently been explored using site-directed mutagenesis [90,91]. The muscle-selective A-kinase anchoring protein (mAkap) assembles a PKA/PDE4D3 signaling module [92]. The *in vitro* anti-inflammatory and immunomodulatory potential of Roflumilast and its *in vivo* efficacy in airway disease models have also been reported [93,94].

Acknowledgements

The authors thank Rick Friesen, Yves Girard, Dave Percival and Brian Kennedy at Merck Prosser for comments and suggestions during the preparation of the manuscript.

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Patents

- P1. Flockner D, Gutterer B, Hatzelmann A, Schudt C, Beume R, Kilian U, Wolf H: Fluoroalkoxy-substituted benzamides and their use as cyclic nucleotide phosphodiesterase inhibitors. 1995, WO 95/01338.
- P2. Nieman R, Rebuck AS, Torphy TJ: Treatment of pulmonary disease e.g. asthma and chronic obstructive pulmonary disease, comprises phosphodiesterase 4 inhibitor and beta adrenergic bronchodilator. 2000, WO 2000/12078.



Cyclic AMP specific phosphodiesterase activity and colon cancer cell motility

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Received 7 May 2001; accepted in revised form 11 June 2001

Key words: cAMP, colon cancer, DLD-1, phosphodiesterase, rolipram

Abstract

To investigate mechanisms for regulation of intracellular cAMP involved in cancer cell invasion, phosphodiesterase (PDE) activity in a colon cancer cell line, DLD-1, was studied. Activities of PDE 2, 4, and 5 were detected in DLD-1 cells by pharmacological approach. Specific and cell permeable inhibitors for those PDEs were used to determine which PDE is responsible for cAMP turnover involved in cancer cell motility. Treatment of DLD-1 cells with rolipram and Ro-20-1724, inhibitors for PDE 4, elevated intracellular cAMP contents three to five times of control. EHNA, an inhibitor for PDE 2, and zaprinast, an inhibitor for PDE 5, did not affect cAMP levels. To assess cellular motility, we utilized chemotaxis assay. EHNA and zaprinast did not suppress serum-induced chemotaxis. In contrast, rolipram and Ro-20-1724, suppressed chemotaxis in a dose dependent fashion. These suggest that PDE 4 plays a critical role in regulating intracellular cAMP levels of colon cancer cells and is involved in cancer invasion. PDE 4 can be a novel target of anti-invasion drug.

Abbreviations: PDE – phosphodiesterase; DMSO – dimethyl sulfoxide; FBS – fetal bovine serum; EHNA – erthro-9-(2-hydroxy-3-nonyl)adenine HCl; DMF – dimethyl formamide

Introduction

Cancer metastasis is a complex event including a number of cellular functions, such as motility, invasion, attachment and proliferation. Among them, cellular motility and invasion are involved in initial steps of metastasis at original sites. Intracellular cAMP has been shown to regulate cellular motility in various cell types *in vitro* [1, 2]. The levels of cAMP is regulated by the balance of adenylyl cyclase and phosphodiesterase (PDE) activities in cells. Although the effects of PDE inhibitors on cell motility were reported in various experimental models [3, 4], no study has been performed to investigate PDE activities that control cAMP levels in colon cancer cells.

The aim of this study was to investigate PDE activities of a colon cancer cell line, DLD-1, and understand how they regulate cancer cell motility. The results obtained will provide useful information for the discovery of a novel anti-metastasis drug.

Materials and methods

Reagents and cell culture

Rolipram, (±)-4-[3-(cyclopentyloxy)-4-methoxy-phenyl]-2-pyrrolidinone, and Ro-20-1724, 4-(3-Butoxy-4-methoxy-benzyl)-2-imidazolidinone, specific and cell-permeable inhibitors for PDE 4, was purchased from BIOMOL Research Labs., Inc. (Plymouth Meeting, Pennsylvania). Zaprinast, 1,4-dihydro-5-(2-propoxyphenyl)-7H-1,2,3-triazol(4,5-d)pyrimidine-7-one, a specific inhibitor for PDE 5, and vinpocetine, a specific inhibitor for PDE 1, were from Sigma (St. Louis, Missouri). EHNA, erthro-9-(2-hydroxy-3-nonyl)adenine HCl, a specific inhibitor for PDE 2 was from BIOMOL Research Labs., Inc. (Plymouth Meeting, Pennsylvania). Cilostazol, a specific inhibitor for PDE 3, was synthesized by Otsuka Pharmaceutical Co., Ltd (Tokushima, Japan). All of these PDE inhibitors were dissolved in dimethyl sulfoxide (DMSO) and the same amount of DMSO was added to the control experiments at the concentration of 0.1% except PDE assays, where dimethyl formamide (DMF) was used as solvent. A human colon cancer cell line, DLD-1, was kindly provided from Drs Kawai and Horimoto (Osaka University Medical School, Suita, Japan) and grown in RPMI1640 supplemented with 10% fetal bovine serum (FBS). The cells at 70 to 80% confluency were trypsinized and used for experiments described below.

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Phosphodiesterase assay

DLD-1 cells were harvested and suspended in 50 mM Tris-HCl, pH 7.5, 1 mM MgCl_2 , 1 mM EDTA, 0.1 mM EGTA, 40% glycerol, and proteinase inhibitor cocktail (Sigma, St. Louis, Missouri). After sonication two times for 10 seconds, the suspensions were centrifuged at 10,000 g for 10 min and the supernatants were used for PDE assays. PDE activity was measured by the method previously described [5]. Reaction buffer contained 50 mM Tris-HCl pH 7.5, 5 mM MgCl_2 , 2 mM EGTA, 0.1 mg/ml bovine serum albumin. Substrate was [^3H] cAMP or [^3H] cGMP at the concentration of 0.1 μM or 3 μM . DLD-1 cell extract in the presence or absence of one of the PDE inhibitors was incubated in the reaction buffer at 30 °C for 10 min. Assays were stopped by incubating the tubes in a 100 °C for 5 min. After cooling, snake venom (0.1 mg/ml) was added to each assay followed by incubation at 30 °C for 10 min. The samples were applied to Bio-Rad AG 50W-X8 resin columns. After washing, the columns were eluted with 1 N NH_4OH and the eluates were collected in scintillation vials. The radioactivity of [^3H] adenosine or [^3H] guanosine was counted by liquid scintillation. All compounds were dissolved in DMF and the final concentration of DMF was 0.5% in the assays.

Measurement of intracellular cAMP levels

The effects of PDE inhibitors on cAMP content in DLD-1 cells were assessed by enzyme immunoassay (EIA). DLD-1 cells were cultured in 96 well cell culture plate at the density of 10,000 cells/well for 14 h at 37 °C in RPMI1640 containing 10% FBS with or without PDE inhibitors. After the incubation, cells were lysed and the cell lysates were used to determine the intracellular cAMP content using a cAMP EIA kit (Amersham Pharmacia Biotech, Piscataway, New Jersey).

Proliferation and chemotaxis assay

DLD-1 cells were cultured in 30 mm dish with RPMI medium containing 10% FBS and one of the PDE inhibitors was added. At day 1, 3, and 5, the cells were trypsinized and cell numbers were counted with Coulter Counter (Beckman Coulter, Miami, Florida). Chemotaxis assay was performed using non-coated transwell polycarbonate membranes (Coster, Cambridge, Massachusetts) with pore size of 8 μm . Lower compartments included 5% FBS as a chemoattractant to induce trans-membrane migration. Cells on the bottom sides of transwells were fixed and stained with Giemsa solution and migrated cells were counted through bright field microscopy. The area of a field through microscopy was about 1 mm^2 .

Results

Phosphodiesterase activities in DLD-1 cell lysates

First, we used a lower concentration of cAMP (0.1 μM) as substrate to determine activities of low K_m cAMP phosphodiesterase isozymes (Figure 1A). The activity without inhibitor was 1.46 pmole/min/ 10^6 cells. A specific PDE 4 inhibitor, rolipram, inhibited this activity in a dose dependent fashion. The IC_{50} for this inhibition was 0.3 μM , which is a reported IC_{50} for purified PDE 4. A specific PDE 2 inhibitor, EHNA, did not inhibit this activity. Cilostazol, a specific PDE 3 inhibitor, showed minimum inhibition. The IC_{50} for the inhibition by cilostazol was relatively high (77.3 μM) and this was about the same as the IC_{50} for the inhibition of purified PDE 4 by cilostazol. These indicated that there is a PDE 4 activity dominant in DLD-1 cells, and that PDE 2 and PDE 3 activities against cAMP were very low if any. When we used a higher concentration of cAMP (3 μM), an activity was 15.5 pmole/min/ 10^6 cells and a similar inhibitory profile was obtained (Figure 1B). This also indicated that PDE 4 is a dominant phosphodiesterase against cAMP in this cell lysate.

Next, we used cGMP as substrate to evaluate activities of cGMP specific phosphodiesterases. When a lower concentration (0.1 μM) of cGMP was used (Figure 1C), an activity was 0.46 pmole/min/ 10^6 cells. A specific inhibitor for PDE 5, zaprinast, inhibited this activity potently (IC_{50} = 0.22 μM). Cilostazol, a PDE 3 inhibitor, also inhibited this activity but the IC_{50} was higher (7.8 μM). As cilostazol is known to have some inhibitory effects on PDE 5 around these concentrations (4–10 μM) [5], these indicated that PDE 5 activity exists in this cell lysate. Vinpocetine, a PDE 1 inhibitor, did not show significant effects indicating the absence of PDE 1 activity. EHNA did not show inhibition in this assay. To estimate cGMP-stimulated phosphodiesterase (PDE 2) activity, higher concentration (3 μM) of cGMP was used (Figure 1D). In this assay, the activity was 5.46 pmole/min/ 10^6 cells and EHNA exerted inhibitory effects (IC_{50} = 4.7 μM). This clearly showed existence of PDE 2 activity against cGMP in this cell lysate. In addition, cilostazol, whose IC_{50} for purified PDE 2 is reported to be 5.6–58 μM [5], inhibited this activity at relatively higher doses (IC_{50} = 15.3 μM). Taken together, we concluded that in DLD-1 cell lysate, there are PDE 2, PDE 4 and PDE 5 activities predominantly existing and that PDE 1 and PDE 3 activities are not detectable in this assay system.

Intracellular cAMP levels in DLD-1 cells treated with PDE inhibitors

To further determine whether PDE 2, 4, and 5 are active and regulating cAMP levels in DLD-1 cells, we next treated the cells for 14 h with specific inhibitors for those PDEs and measured intracellular cAMP levels by immunoassay (Figure 2). Rolipram and Ro-20-1724, inhibitors for PDE 4, increased intracellular cAMP significantly (vs. DMSO, $P < 0.01$, Student's t -test). In contrast, zaprinast-treatment did not affect intracellular cAMP levels. Since zaprinast is

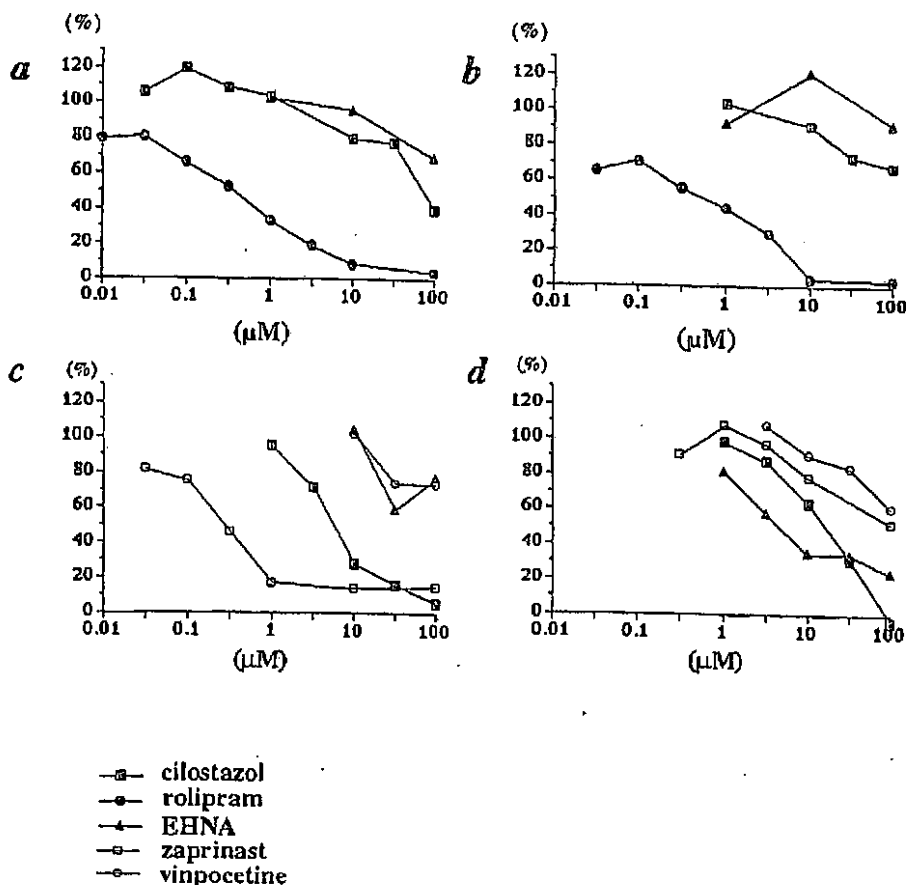


Figure 1. Phosphodiesterase activity of DLD-1 cell lysates. % inhibition of control activity was plotted. *a*, Lower concentration (0.1 μ M) of cAMP as substrate. *b*, higher concentration (3 μ M) of cAMP as substrate. *c*, lower concentration (0.1 μ M) of cGMP as substrate. *d*, higher concentration (3 μ M) of cGMP as substrate. Cilostazol (closed rectangle), rolipram (closed circle), EHNA (closed triangle), zaprinast (open rectangle), vinpocetine (open circle).

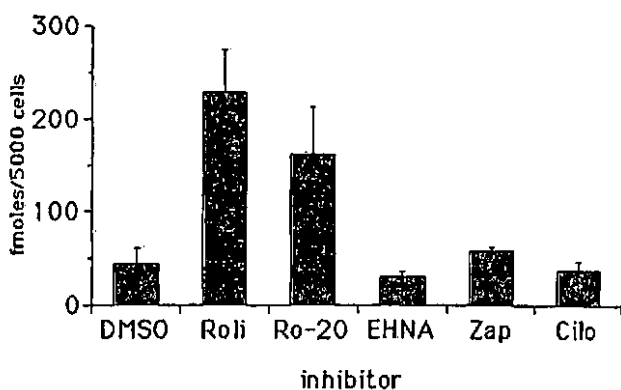


Figure 2. Intracellular cAMP levels of DLD-1 cells treated with various phosphodiesterase inhibitors. Values are the mean of triplicate wells ($\pm$ SE). DMSO: dimethyl sulfoxide, Rolipram (50 μ M), Ro-20: Ro-20-1724 (50 μ M), EHNA: erthro-9-(2-hydroxy-3-nonyl)adenine (50 μ M), Zap: zaprinast (50 μ M), Cilo: cilostazol (50 μ M).

an inhibitor for cGMP specific phosphodiesterase (PDE 5), this confirmed the results from *in vitro* PDE activity study. Cilostazol, a specific inhibitor for PDE 3, did not increase intracellular cAMP levels and this also confirmed the data of PDE assay. EHNA had no effect on cAMP levels although PDE 2 activity was detected in cell lysate. This indicated that

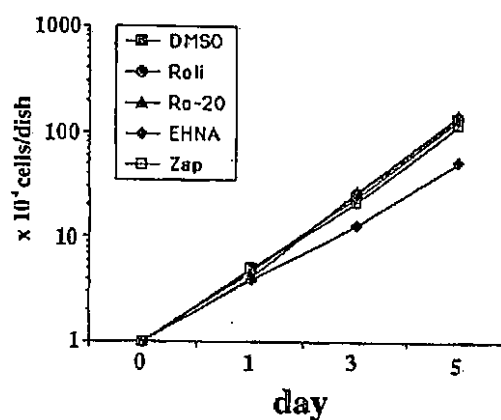


Figure 3. Effects of phosphodiesterase inhibitors (50 μ M) on cell growth. Cell numbers on 30 mm dishes were plotted. DMSO (closed rectangle), rolipram (closed circle), Ro-20-1724 (closed triangle), EHNA (closed diamond), zaprinast (open rectangle). Values are the mean of cell numbers from three culture dishes ($\pm$ SD).

cGMP levels are not high enough to activate PDE 2 in intact DLD-1 cells of this condition.

The effects of PDE inhibitors on proliferation of DLD-1 cells

Before performing cellular motility assay, we checked the cellular toxicity of PDE inhibitors. All the inhibitors used in this study did not cause any toxicity at 50 μ M within 48 h, which was confirmed by trypan blue staining. Although we did not detect any cell toxicity in 48 h incubation, the effects of these PDE inhibitors on cellular growth of DLD-1 were of interest. The cells were incubated in 5% FBS medium with or without PDE inhibitors at 50 μ M (Figure 3). PDE 4 inhibitors, rolipram and Ro-20-1724 did not show any effect on cell proliferation up to day 5. Zaprinast inhibited cell proliferation in a minimum range. In contrast, EHNA, an inhibitor for PDE 2, suppressed cell growth significantly at day 3 and 5 (vs. DMSO, $P < 0.01$, Student's t -test). The cells under this condition did not die even at day 5. The inhibitory effects of EHNA on cell growth seemed to be caused not by cellular toxicity but by PDE 2 inhibition for a long incubation time.

The effects of PDE inhibitors on chemotaxis of DLD-1 cells

Because the increase of cAMP in cancer cells has been shown to suppress cellular motility [2], we designed experiments to investigate the effects of PDE inhibitors on DLD-1 cells. Two chamber system was applied to estimate chemotaxis of DLD-1 cells (Figure 4). The cells were seeded onto the upper chamber and 5% FBS was used in the lower chamber as a chemoattractant. PDE inhibitors were applied both in upper and lower chambers at the same concentrations. After 10 h, DLD-1 cells migrated through the pores and spread out on the bottom sides of the transwells (Figure 4). Rolipram (50 μ M) and Ro-20-1724 (50 μ M), inhibitors for PDE 4, suppressed chemotaxis induced by FBS. Zaprinast (50 μ M) and EHNA (50 μ M) did not significantly affect it. The cells on the bottom sides were counted (Figure 4G). Rolipram and Ro-20-1724 suppressed chemotaxis of DLD-1 induced by FBS significantly in a dose dependent manner (vs. DMSO, $P < 0.01$, Student's t -test). Cilostazol (50 μ M) suppressed chemotaxis (vs. DMSO, $P < 0.01$, Student's t -test) as we reported previously [4], although intracellular cAMP was not elevated in the condition used. We tested another colon cancer cell line, WiDr, that has relatively high activity of PDE 4 and observed similar suppressive effects on cellular migration by rolipram (Murata et al., unpublished results).

Discussion

Cyclic AMP has been demonstrated to be one of the important second messengers to regulate cellular motility. In 1983, Mokashi et al. reported that drugs to elevate cAMP levels inhibit chemotaxis of tumor cells [2]. To maintain optimal levels of cAMP in cells effectively, phosphodiesterases (PDEs) are important regulators as well as adenylyl cyclase. In this report we investigated PDEs in colon cancer DLD-1

cells and by using specific inhibitors we found that PDE 4 is involved in cancer cell motility.

PDEs are classically categorized into seven classes [6]. PDE 1 is called Ca²⁺/calmodulin dependent PDE. PDE 2 can be stimulated by cGMP and PDE 3 can be inhibited by cGMP. PDE 4, also called as a cAMP-specific and rolipram-sensitive PDE, has four subtypes, i.e., PDE 4 A, B, C, D. Each enzyme has its splicing variants. PDE 5, cGMP specific PDE, is involved in smooth muscle relax. PDE 6 is photoreceptor cGMP PDE. PDE 7 is cAMP specific but insensitive to rolipram or IBMX, whose physiological roles are not fully elucidated yet. Recently, novel subtypes of PDEs were cloned and characterized [7–11], but biological roles of these novel types of PDEs are not elucidated because of lack of specific inhibitors. We took advantage of using specific inhibitors for PDE 1 to 5 in order to clarify which PDE is dominantly active in DLD-1 cell lysate. Although activities of PDE 6 to 11 could not be definitely assessed, the data indicated that PDE 4 family is dominantly active in DLD-1 cells.

PDE 4 and its significance in cancer cell function have been well characterized by using a specific inhibitor, rolipram [12]. It was shown that higher activity of PDE 4 in malignant carcinoma than its counterpart non-malignant cells [13]. In virus-transformed lymphocytes, PDE 4 activity was up-regulated [14]. Chemotactic migration was demonstrated to be enhanced by rolipram-sensitive PDEs in a breast cancer cell line [3]. Integrin $\alpha 6 \beta 4$ was shown to decrease intracellular cAMP concentrations by stimulating rolipram-sensitive PDEs. They also showed that activation of rho through integrin $\alpha 6 \beta 4$ was suppressed by IBMX, a non-specific PDE inhibitor [15]. Although our data supported this concept, it is still unknown which subtype of PDE 4 is involved in cellular motility. Iona et al. established specific antibodies against each subtype of PDE 4 [16]. Further investigation using those antibodies will clarify which subtype of PDE 4 is dominant in cancer cells or tissues.

Although we did not detect significant suppression on proliferation or viability of DLD-1 cells by rolipram incubation, induction of apoptosis by a PDE 4 inhibitor was reported in different cell lines. In chronic lymphocytic leukemia cells, PDE 4A and B were identified [17]. They demonstrated successful induction of apoptosis of leukemia cells by rolipram and proposed that rolipram can be a novel therapeutic target of malignant diseases. Anti-proliferative effects of PDE inhibitors were also reported [18] whereas the effects may not be associated with PDE inhibition [19]. PDE 4 has four subtypes and rolipram suppressed all of them. Each of PDE 4 subtypes may have a specific role in cellular function. The subtype of PDE 4 predominantly active in DLD-1 cells, probably PDE 4C or D, may be involved in cellular motility rather than proliferation.

The inhibitory effects of PDE 2 inhibitor, EHNA, on cell growth were moderate but of interest. Although we could not detect elevation of cAMP levels in DLD-1 cell lysate by EHNA treatment, it is possible that local accumulation of cAMP in cells by specific PDE 2 inhibition may contribute to the partial and moderate suppression of

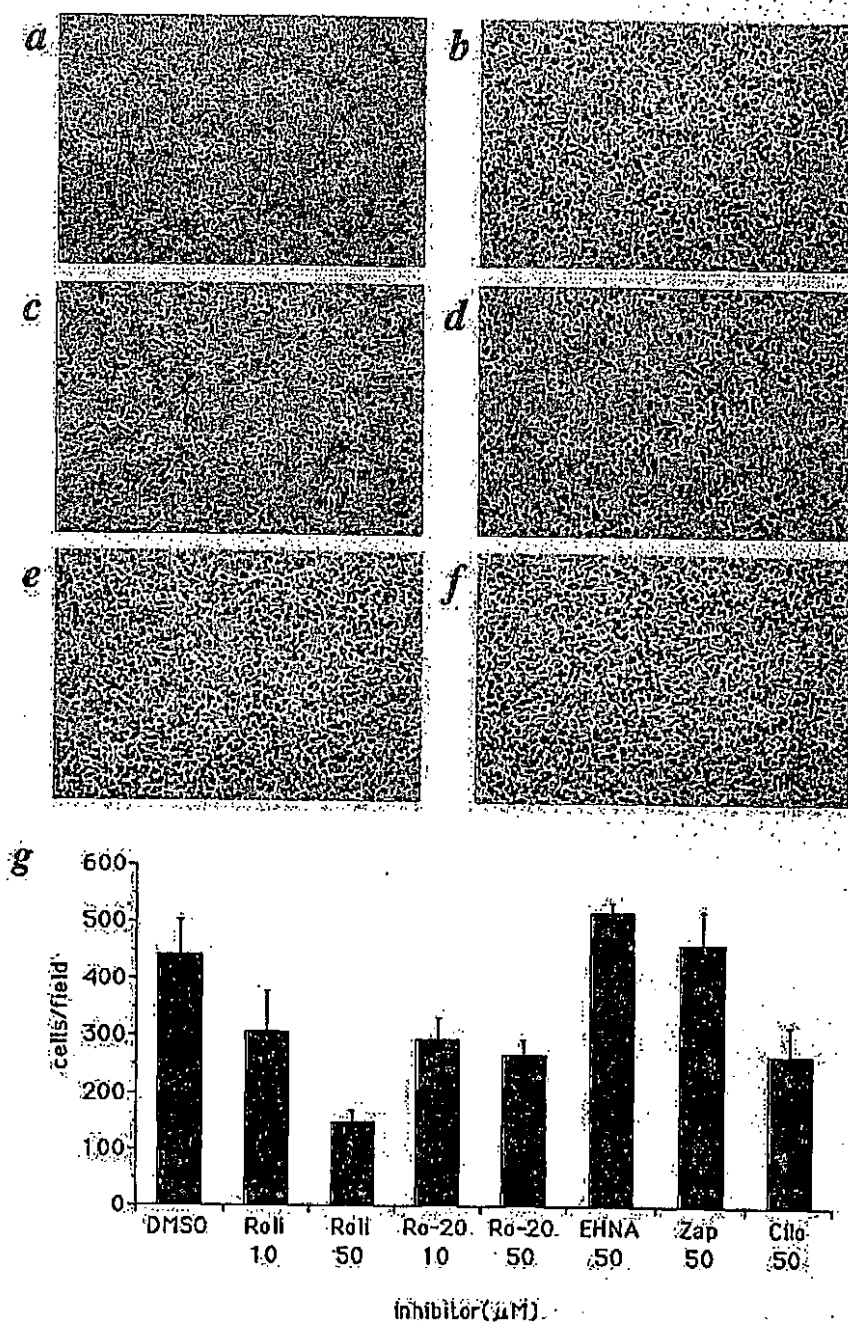


Figure 4. Effects of phosphodiesterase inhibitors on FBS-induced chemotaxis of DLD-1 cells. a, DMSO without chemoattractant, b, DMSO, c, rolipram (50 μ M), d, Ro-20-1724 (50 μ M), e, EHNA (50 μ M), f, zaprinast (50 μ M). g, Cells were counted through microscopy. Results are representative of three independent experiments, and indicated values represent the mean of three fields counted ($\pm$ SD). DMSO: dimethyl sulfoxide, Roli: rolipram, Ro-20: Ro-20-1724, EHNA: entho-9-(2-hydroxy-3-nonyl)adenine, Zap: zaprinast, Cilo: cilostazol.

cell growth. Further investigation needs to be performed to clarify the mechanisms.

In the previous report, we showed that a specific inhibitor for PDE 3, cilostazol, suppresses colon cancer cell motility using a variety of *in vitro* experimental models [4]. We also confirmed suppressive effects of cilostazol on chemotaxis of DLD-1 in this report (Figure 3G). However, we analyzed pharmacologically PDE activities of colon cancer DLD-1 cells and found that there is no detectable activity of PDE 3 in those cells. In addition, cilostazol did not elevate intracellular cAMP levels. The question whether the suppression

of cell migration by cilostazol comes from the inhibition of PDE 3 or not remains to be solved. It is yet possible that undetectable increase of local cAMP in particular cellular components by inhibition of PDE 3 by cilostazol could cause moderate PKA activation and significant suppression of cellular motility. In fact, in rat mesangial cells, cilostazol showed anti-mitogenic effects with PKA activation without detectable increase of cAMP [20].

Receptor-mediated suppression of adenylyl cyclase through hetero-trimeric G protein (G_i) and down-regulation of cAMP have been proposed as one of the mechanisms to

control cellular motility. A number of studies have been reported to support this concept including our laboratory using different cell types and stimuli [21, 22]. However, keeping effectively intracellular cAMP levels lower can be done by activation of phosphodiesterases. Activation of PDE 4 in cancer cells and consequent hydrolysis of cAMP might be another mechanism for cancer cell invasion.

Our data suggested that PDE 4 can be a novel target of cancer therapy. PDE 4 inhibitors including rolipram and Ro-20-1724 have been studied as anti-depressant drugs in humans but did not become tolerable because of their side effects on central nerve system [24]. Although a number of studies showed anti tumor effects of PDE 4 inhibitors *in vitro* [3, 17–19], nobody reported their effects on experimental cancer models. The efficacy of PDE 4 inhibitors on animal metastatic models should be investigated in the next stage of our project. Recently, second generation PDE 4 inhibitors have been introduced and some of them have been already applied for clinical use as anti-asthma or anti-inflammatory drugs [25, 26]. Those inhibitors might be useful as anti-invasion therapy for colon cancer patients.

Acknowledgements

The authors thank Mrs F. Fukui and Ms H. Sugiura for excellent technical assistance.

This work was supported by grants from the Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare (to M.K. and M.M.).

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The potential of PDE4 inhibitors in asthma or COPD

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Current Opinion in Investigational Drugs 2000 1(2):204-213
© PharmaPress Ltd ISSN 0967-8298

At least 11 families of distinct phosphodiesterase (PDE) isoenzymes are known to regulate the function of many cells secondary to altering the intracellular levels of second messengers including cyclic 3'-5'-monophosphate (cyclic AMP) and cyclic 3'-5'-guanosine monophosphate (cyclic GMP). While there is a wide distribution of these enzymes throughout the body, it is of interest that inflammatory cells thought to participate in the pathogenesis of inflammatory diseases including asthma and chronic obstructive pulmonary disease (COPD), preferentially express PDE4. This finding has stimulated the search for highly selective inhibitors of these enzymes. Unfortunately, PDE4 inhibitors tend to be associated with a number of unwanted side effects including headache and emesis. However, attempts have been made through rational drug design to synthesize compounds that demonstrate improved side effect profile. Such drugs offer an exciting opportunity to selectively downregulate inflammatory cell function as a novel therapeutic approach in the treatment of airway disease.

Keywords Phosphodiesterase inhibitors, COPD, PDE4, asthma

Introduction

At least 11 families of PDE, including PDE1-7 [1] and PDE8-11 [2] are known to exist, based upon a variety of criteria including substrate specificity, inhibitor potency, enzyme kinetics and amino acid sequence. These enzymes are distributed widely throughout the body, differentially expressed in cells and localized to different compartments within cells. The functional significance of the subcellular localization of PDEs is not completely understood, although there is a considerable body of evidence to suggest that the

expression of PDE in cellular domains can tightly regulate the levels of cyclic nucleotides in the vicinity of effector proteins and are therefore implicated in the regulation of cell function [3].

The development of selective PDE inhibitors has facilitated our understanding of the role that these enzymes play in cell biology. The selective targeting of individual PDE isoenzymes has profound implications for the treatment of disease, as recently highlighted with the introduction of the PDE5 selective inhibitor, sildenafil (Pfizer), for erectile dysfunction [4]. Many pharmaceutical companies are attempting to discover the 'Viagra of the airways' for diseases like asthma and COPD. In this context, PDE4 has been selectively targeted using chemical inhibitors on the basis of the clinical efficacy of the archetypal non-selective PDE inhibitor, theophylline, which has long been used in the treatment of asthma and COPD. A number of highly potent PDE4 inhibitors have been tested in clinical trials and shown to have therapeutic potential. However, one of the major stumbling blocks to the development of these inhibitors is the potential side effect profile, including emesis that is a characteristic feature of many of these drugs, although attempts are being made to reduce these unwanted side effects. This review will highlight the current state of play with regard to the potential use of these drugs in the treatment of asthma and COPD.

Properties and classification of PDE4

PDE4 is a cyclic AMP specific isoenzyme ($K_m = 0.2$ to 4 mM), showing very low affinity for cyclic GMP ($K_m = > 1000$ mM), the latter without effect on PDE4 catalytic activity. Four PDE4 subtypes (PDE4A to D) have been cloned and expressed, with additional complexity arising as a consequence of mRNA splicing resulting in isoforms with alterations in amino acid sequences within the N terminal region [3]. In order to gain insights into the functional significance of PDE4, various studies investigated the distribution of PDE4. It is clear that PDE4C is predominantly localized to the testis, skeletal muscle and human fetal lung [5], while PDE4A, B and D are known to be distributed in many inflammatory cells in man (Table 1).

Table 1. Summary of the expression of mRNA for PDE4 genes in human cells.

Cell type	A	B	C	D	Reference
CD4 T cell	+	++ (B2)		Weak	[51]
Th1 cells	++	++	-	-	[122]
Th2 cells	++	++	-	++	[122]
CD8 T cell	++	++ (B2)		++	[51]
B cell	++	++ (B2)		++	[123]
Monocyte	+	++ (B2)	-	Weak	[25,51,124,125]
Eosinophil	++	++	-	++	[126,51]
Neutrophil	±	++ (B2)	-	±	[125,126]
Macrophage	++				[16]
Brain	++	++	±	++	[5,126]*
Area postrema				++	[45] (44)**
Epithelium	+ (A5)		+ (C1)	+ (D2) + (D3)	[127] [128]

*Analysis performed in rat brain. **Immunohistochemical detection using mouse brain. Text in parenthesis denotes splice variant. + and - denote presence and absence of expression, respectively.

Analysis of the amino acid sequence of PDE4 revealed a catalytic domain and two upstream conserved regions (UCRs) that are unique to this family of PDE. Using deletion analysis, studies have shown that the catalytic domain in PDE4A4B, for example, lies between the amino acid sequences 332/365 to 680/772 [3,6••]. Similarly, PDE4B2B has a catalytic domain between amino acid sequence 152-528 [7••,8••]. The atomic structure of the catalytic domain of 4B2B has recently been published, showing important structural features within the binding pocket for cyclic AMP including the presence of two metal ions, most likely zinc and magnesium, that are important for binding the cyclic phosphate group and various other amino acids critical for cyclic AMP binding [9••].

The cDNAs for PDE4 encode for enzymes that can exist as either the long form, containing both UCR regions, and a short form, characterized by either a lack in UCR1 and intact or partially truncated UCR2 region. It is thought that the short and long forms differ with respect to enzyme activity, subcellular localization and activation by different intracellular signaling pathways. Thus, PDE4D3 catalytic activity is increased by a protein kinase A-dependent mechanism [10••], a consequence of phosphorylation of Ser54 [11••]. Additionally, specific sites in the UCR region are also subject to phosphorylation by MAP kinase-dependent mechanisms which could have important implications for PDE4 activity [3]. Furthermore, the N terminal region is implicated in targeting PDE4 to specific domains within the cell by virtue of protein-protein interactions with SH3 domain-containing proteins [12••]. Regions near the carboxyl terminus are also implicated in the regulation of PDE4 function. Thus, substitution of Ser487 for Ala resulted in a significant attenuation of MAP kinase-dependent phosphorylation of PDE4B2B [7••]. Similarly, phosphorylation by ERK2 kinase of PDE4D3 at Ser579 in the carboxyl terminal region, led to a significant reduction in catalytic activity [13••].

Further complexity arises with findings that alteration in the activity of PDE4 by ERK2 kinase is also influenced by the presence of UCR regions. Thus while phosphorylation of PDE4D3 at Ser579 resulted in a reduction in cyclic AMP PDE activity, an increase in catalytic activity was observed following phosphorylation of Ser491 in PDE4D1, a PDE4 enzyme that lacks a UCR1 domain [14••]. These findings suggest that different splice variants of PDE4 may be differentially regulated by intracellular signaling pathways that may have important implications in the regulation of cell function under normal physiological and pathophysiological conditions. Indeed, alterations to the N terminal regions of these proteins have important functional consequences as this may alter their subcellular localization [12••], activation [7••,10••,15] and inhibition by PDE4 inhibitors [10••]. Moreover, the observation of alterations in PDE4 expression during cell differentiation [16] or following activation by cytokines, growth factors and lipid mediators [7••,17-19] could have important functional consequences during an inflammatory episode.

It has long been recognized that the archetypal PDE4 inhibitor, rolipram (Schering AG), binds with high affinity to brain tissue compared with peripheral organs [20] yet is at least 2- to 3-fold less potent at inhibiting PDE4 catalytic

activity in this tissue [21]. The significance of this discrepancy was later clarified in studies expressing human recombinant PDE4 in yeast and showing that the high affinity rolipram binding site and the PDE4 catalytic domain reside on the same gene product [22••,23]. Interestingly, there was little correlation between the ability of a range of compounds to displace rolipram binding from PDE4 and their ability to inhibit PDE4 catalytic activity, raising the possibility of synthesizing compounds that could selectively target these sites. The functional significance of the two domains recognized by PDE4 inhibitors was clarified further in studies examining rolipram binding and PDE4 catalytic activity in N terminally-truncated enzymes expressed in yeast, COS and Sf9 cells [6••,8••,24••]. Specific regions within the N terminal domain of PDE4A are important for determining high affinity binding by rolipram and the removal of this site from the protein did not abolish catalytic activity nor the ability of rolipram to inhibit PDE4 catalytic activity, suggesting that binding to the high affinity site is not a prerequisite for inhibition of catalytic activity [6••,24••]. Similarly, expression of an N terminal-truncated PDE4B2B (152-564) resulted in a protein which lacked a high affinity binding site for rolipram compared with PDE4B2B (81-564) [8••], suggesting that specific sequences within the N terminal domain are necessary for the expression of high affinity binding. However, the binding of another PDE4 inhibitor, RP-73401 (Rhône-Poulenc), to PDE4A was unaffected by the loss of this specific amino acid sequence within the N terminal domain, but the ability of rolipram to displace RP-73401 binding was characterized by a two-site binding model [6••]. The implication of these findings is that specific amino acid sequences outside the catalytic domain of PDE4 can alter the conformation of the protein, such that it binds rolipram with high affinity and therefore the 'high affinity' binding site represents a different conformation of the same protein [6••,8••,22••,24••]. There is biochemical evidence supporting the view that PDE4 can exist in different conformational states, as different methods employed to isolate PDE4 from cells can lead to differences in catalytic activity and inhibitor sensitivity [25,26]. A number of intracellular processes including phosphorylation [7••,10••,11••,13••,14••] or the presence of co-factors, such as magnesium ions [27], are known to alter PDE4 catalytic activity.

Pharmacological studies have been employed in order to determine structure-activity relationships between different PDE4 inhibitors and a number of functional studies have shown correlation between PDE4 inhibitor potency and the ability of PDE4 inhibitors to inhibit various aspects of cell function or rolipram binding. The ability of PDE4 inhibitors to inhibit gastric acid secretion [28], emesis [29], fMLP-induced myeloperoxidase release from human neutrophils [30], inhibition of purified solubilized PDE4 from guinea pig eosinophils and potentiation of isoprenaline-induced cyclic AMP accumulation from guinea pig eosinophils [31], correlated with the ability of these inhibitors to displace high affinity rolipram binding. In contrast, the ability of compounds to inhibit PDE4 catalytic activity correlated with the potency of these agents against LPS-induced TNF α release by human monocytes [25,30], fMLP-induced superoxide production by guinea pig eosinophils [28] and interleukin (IL)-2 release by murine splenocytes [32]. The possibility that PDE4 may exist as different conformers has

been used in an attempt to discover novel inhibitors that are selective for the 'low' affinity conformer as this subtype is suggested to be responsible for regulating cell function, while the 'high' affinity conformer is linked to the side effect profile seen with PDE4 inhibitors.

The ability of PDE4 inhibitors to activate emetic centers within the CNS may be a consequence of a peripheral action of these drugs secondary to raising intracellular levels of cyclic AMP in gastric acid secreting cells and/or afferent neurons in the gut. Alternatively, stimulation of the area postrema, a region within the CNS with a poorly developed blood brain barrier and therefore accessible to substances within the circulation, can lead to activation of the emetic center within the CNS [29,33]. Since emesis and gastric acid secretion correlate with the potency of PDE4 inhibitors to displace rolipram binding (high affinity PDE), this led to the suggestion that drugs with low affinity for this site may be useful in improving the side effect profile of these drugs [28,30]. However, some aspects of cell function may also correlate with inhibitors that target the 'high' affinity conformer and suggest that this method may be of limited value for the future development of PDE4 inhibitors with low emetic potential [30]. It is therefore of interest that CDP-840 (Celltech) [34] and SB-207499 (SmithKline Beecham) [35] demonstrate a 'high' to 'low' ratio of 5 and 1.3, respectively. In contrast, rolipram is one to two orders of magnitude more selective for the high affinity binding site compared with inhibition of PDE4 catalytic activity [6,22,34,35]. Accordingly, both compounds have low emetic potential and document low side effect profiles in asthma [35,36,37]. SB-207499 inhibited myeloperoxidase release from human neutrophils with equal potency to rolipram, despite the acknowledgement that this particular cell function is modulated by the 'high' PDE4 conformer [30,38], suggesting that additional factors may also govern why these drugs demonstrate a better side effect profile compared with other PDE inhibitors. SB-207499 is negatively charged at normal pH that may retard its ability to gain access to the area postrema, although clearly not enough to retard access across inflammatory cells [37,38]. It is unclear whether the expression of splice variants of PDE4 in different cells also contribute to the observed correlations between cell function and PDE4 inhibitor potency or high affinity rolipram binding because of the similarities in the expression of PDE4 subtypes in these cells (Table 1) and the lack of subtype selectivity of the PDE4 inhibitors tested in these studies.

Another approach that is being investigated is whether compounds can be synthesized that are selective for different PDE4 subtypes in an attempt to diminish the side effect profile and selectively target inflammatory cells. While CDP-840 does not demonstrate subtype selectivity for PDE4A, B and D [34], SB-207499 shows 5-fold selectivity toward PDE4D compared with the other two subtypes [39,40]. SB-207499 is considerably less emetic than rolipram and is well tolerated by subjects, although it is not free from emesis. Therefore, there is clearly a need to discover highly potent PDE4 inhibitors with little if any side effect profile. Consequently, a number of compounds have been synthesized that demonstrate selectivity for either PDE4A/B or PDE4D with a difference of up to 55-fold [39,40] and structure-activity relationships have been documented.

Thus, a significant correlation was found between PDE4A/B inhibitory potency and inhibition of TNF α release from monocytes, proliferation of T lymphocytes and oxidative burst from human eosinophils. In contrast, no significant correlation between PDE4D inhibitory potency and inhibition of lymphocyte proliferation and TNF α release from monocytes was observed, which was consistent with the finding of weak PDE4D expression in these cells [39] (Table 1). However, a correlation was observed against human eosinophil function and selectivity for PDE4D, which was consistent with the presence of PDE4D in these cells [40]. Therefore, it may be possible to synthesize compounds that document greater subtype and cell selectivity. An important question that needs to be addressed is whether selective targeting of PDE4 subtypes will be sufficient to modulate inflammatory cell function, particularly if cells contain multiple PDE4 subtypes.

Pharmacokinetic considerations notwithstanding, there is some evidence that selective targeting of PDE4D significantly improved the ability of compounds to attenuate pulmonary eosinophil recruitment following antigen provocation in allergic rats [40]. In contrast, mice lacking the ability to express PDE4D have impaired growth and fertility, underlying the importance of cyclic AMP signaling in these processes [41]. Of particular interest, however, was the lack of effect of this gene disruption on lymphocyte proliferation, IgE production, IL-4 production and eosinophil recruitment to the airways in a model of murine inflammation [42], features which are characteristic of an allergic phenotype. This contrasts with the findings that the PDE4 inhibitor, rolipram, inhibited allergen-induced eosinophilia in a murine model of airway inflammation [43]. The lack of effect of this gene disruption upon eosinophil recruitment suggests redundancy concerning PDE4 regulation of cyclic AMP signaling in inflammatory cells or alternatively, other PDE4 subtypes play a greater role in regulating allergic inflammation [39].

It remains to be established whether drug targeting of PDE4A/B offers the advantage of suppressing inflammatory cell function *in vivo*, while exhibiting a low emetic profile, considering that PDE4D is expressed in the area postrema in rat and mouse [44,45].

PDE expression in allergic disease

Atopic dermatitis

It had long been recognized that the ability of lymphocytes to raise levels of intracellular cyclic AMP is impaired in mild, severe atopic eczema [46,47] and atopic dermatitis [48,49]. This finding was all the more significant since, unlike asthma, none of the subjects were taking β -adrenoceptor agonist medication. Therefore, tachyphylaxis of β adrenoceptors is not an issue and not a confounding factor in this disease. The increased level of cyclic AMP in mononuclear cells from patients with atopic dermatitis was a consequence of increased cyclic AMP PDE activity as assessed biochemically [49]. The exact splice variant responsible for this activity remains to be established, but evidence was provided of a putative monocyte derived PDE in atopic dermatitis that had increased cyclic AMP PDE catalytic activity and was Ca²⁺/calmodulin- and Ro-201724- (Roche) sensitive [50]. However, such changes in cyclic

AMP PDE activity have not been observed in all studies in cells from patients with atopic dermatitis [51] and it is unclear whether differences in methodology and/or patient selection account for this discrepancy. It has been proposed that this alteration in PDE activity is responsible for the pathogenesis of this disease since a functional consequence of increased cyclic AMP PDE activity in atopic dermatitis include increased IgE production by B lymphocytes, increased histamine and LTC₄ release from basophils, increased IL-4 and reduced IFN γ and IL-10 release by mononuclear cells; physiological responses that can be inhibited by PDE4 inhibitors [52].

An interesting feature of the altered cyclic AMP PDE activity in atopic dermatitis is increased susceptibility of the isoenzyme to inhibition by PDE4 inhibitors. This is reflected by increased inhibitor potency against cyclic AMP catalytic activity [50,53,54] and increased inhibitor potency against the proliferation of mononuclear cells [55]. Interestingly, the antiproliferative effect of theophylline was not altered in atopic dermatitis. The molecular mechanism(s) responsible for the increased PDE catalytic activity and increased sensitivity to PDE4 inhibitors in atopic dermatitis remain to be established, but conformational changes in PDE4 protein [31], phosphorylation of PDE4 [12] and/or expression of a splice variant/novel PDE4 [50] could explain such a phenomenon.

Few studies have investigated the effect of methylxanthines in atopic dermatitis. Mononuclear cells from atopic dermatitis have increased cyclic AMP PDE activity that is more susceptible to PDE4 inhibitors and is reflected by increased PDE4 inhibitor potency [53]. However, cyclic AMP PDE activity in mononuclear cells was restored to normal values in atopic dermatitis subjects who were taking theophylline to control their asthma [53]. An interpretation of this finding is that prolonged treatment with theophylline altered the activity of cyclic AMP PDE activity to control values. This could be attributed to the anti-inflammatory effect of theophylline resulting in a reduction in the release of inflammatory mediators and cytokines known to increase the activity and expression of PDE4. In a double-blind study, the PDE4 inhibitor, Ro-201724, improved psoriatic lesions [56], and daily topical treatment with CP-80633 on one arm improved clinical scores (erythema, induration and excoriation) compared with the untreated arm [54] and indicated the potential use of PDE4 inhibitors in the treatment of atopic dermatitis.

Asthma

Despite our understanding of the mechanisms that can alter PDE4 activity, there is a paucity of data concerning any alteration in the function or expression of PDEs in airway diseases like asthma. The ability of lymphocytes to raise intracellular cAMP and/or increase adenylyl cyclase activity in response to various stimuli including isoprenaline, sodium fluoride and guanylyl-5-yl-imido-biphosphate (GppNHp) was compromised following antigen challenge in atopic asthmatics [57,58]. Similarly, the capacity of alveolar macrophages to raise intracellular cAMP to histamine, salbutamol, PGE₂ and 1-methyl-3-isobutylxanthine (IBMX) was reduced in asthmatics [59,60]. These studies suggest that PDE activity may be increased in inflammatory cells in asthma. However, there is no difference in PDE4 activity in alveolar macrophages [61] and

eosinophils [62,63] noted between atopic and non-atopic subjects. We documented a 50% increase in total cyclic AMP PDE activity in monocytes isolated from asthmatic subjects compared to healthy individuals [64], a finding that is supported by a previous study in atopic dermatitis subjects with a history of airway disease [65].

While there is a considerable body of evidence of increased PDE4 inhibitor sensitivity observed in inflammatory cell populations obtained from some [50,55] but not all [51,62] subjects with atopic dermatitis, we have failed to document a similar finding in monocytes from mild asthmatic subjects [64]. This is consistent with a study examining the potency of Ro-201724 against zymosan-induced release of glucuronidase from neutrophils obtained from asthmatic subjects [66]. Similarly, atopic subjects with upper and/or lower airway disease, and who did not suffer from atopic dermatitis, failed to document an increase in PDE4 inhibitor sensitivity against mononuclear cell proliferation [67,68]. In contrast, PDE4 inhibitors attenuated TNF α and IL-10 release from mononuclear cells stimulated by mitogen to a greater extent in atopic rhinitis compared with healthy subjects [69].

Together, these studies highlight an important observation that alterations in PDE4 catalytic activity or inhibitor sensitivity is dependent upon the type of allergic disease under study and extrapolating from atopic disease other than asthma should be made with caution. The increase in total PDE activity observed in our study was unrelated to an increase in PDE4 activity and is consistent with a lack of evidence of an alteration in the expression of mRNA for PDE4A, B and D in monocytes from mild asthmatic subjects [70]. Thus, despite the lack of evidence of an alteration in PDE4 activity in mild asthma, the rational basis for drug targeting PDE4 in the treatment of respiratory disease stems from the finding that inhibitors of PDE4 can downregulate inflammatory cell function and the proven clinical efficacy of theophylline (see below).

Effect of PDE4 inhibition on cell function

The pharmacology of PDE4 inhibitors has been reviewed in detail elsewhere and these drugs can inhibit the function of a wide variety of cells thought to participate in inflammation including, human eosinophils, neutrophils, monocytes, mast cells, T lymphocytes, macrophages, basophils and airway smooth muscle [1]. It is therefore perhaps not surprising that PDE4 inhibitors can attenuate the inflammatory response in a number of animal models of allergic inflammation. Thus, allergen-induced eosinophil recruitment to the airways, T lymphocyte activation and bronchial hyperresponsiveness are all inhibited by PDE4 inhibitors [71]. Based upon these findings, pharmaceutical companies have developed a range of PDE4 inhibitors that have entered clinical trials with moderate success.

PDE inhibitors in airway disease

Characteristic feature of asthma and COPD

The incidence of respiratory diseases like asthma and COPD continues to increase, despite the availability of current treatment modalities and there is a need to improve our understanding of the pathophysiology of these diseases for the development of novel therapeutic agents. While the exact causes of asthma and COPD are not completely understood, it is clear that both diseases are characterized by

inflammation of the airways and a decline in respiratory function. In asthma, a number of inflammatory cells are thought to contribute toward the pathogenesis of this disease including eosinophils [72] and CD4+ T lymphocytes [73], while it is thought that CD8+ lymphocytes [74] and neutrophils [75] play an important role in COPD. Another important feature of these diseases is the presence of airway wall remodeling and there is evidence of hyperplasia/hypertrophy of airway smooth muscle, increased collagen deposition beneath the basement membrane, increased mucus production, angiogenesis and alterations in extracellular matrix in asthma [76]. In COPD, there is evidence of mucus gland hyperplasia, increased bronchiolar smooth muscle hypertrophy, fibrosis of the small airways and in the case of emphysema, destruction of alveolar tissue [77]. The mainstay of treatment for asthma including bronchodilators like β_2 -adrenoceptor agonists and glucocorticosteroids while for COPD, ipratropium bromide and β_2 -adrenoceptor agonists are used. Reducing the inflammatory response in the airways is thought to be critical in reversing many of the changes seen in these airway diseases. It is therefore of interest that drug targeting of PDE4 isoenzyme may be of potential benefit in these disorders.

Theophylline; archetypal non-selective PDE inhibitor

In 1886, Henry Hyde Salter described the efficacious use of strong coffee taken on an empty stomach as a treatment for asthma [78]. Theophylline is well known for its bronchodilator activity, although it is becoming increasingly apparent that this drug also possesses anti-inflammatory and immunomodulatory activity. The intravenous administration of theophylline and enprofylline prior to allergen challenge inhibited the development of the late asthmatic response [79,80] without any effect on the acute bronchoconstrictor response and associated bronchial hyperresponsiveness to methacholine [81]. Thus, neither functional antagonism of airway smooth muscle shortening nor mast cell degranulation accounted for the attenuated late asthmatic response by theophylline and enprofylline, although in allergic rhinitis, one-week treatment with theophylline reduced histamine release during pollen exposure [82] which indicated that theophylline inhibited mast cell and basophil degranulation in this disorder.

Individuals exposed for long periods of time to certain industrial chemicals develop asthma-like symptoms that can be duplicated in the clinical laboratory following aerosol challenge with the inciting agent. Thus, susceptible individuals demonstrate acute bronchospasm, late asthmatic responses and bronchial hyperresponsiveness following inhalation of toluene di-isocyanate (TDI) [83]. The inflammatory nature of this response has been confirmed by its sensitivity to inhibition by the glucocorticosteroid, beclomethasone. Theophylline partially modified the acute response and attenuated the late asthmatic response induced by TDI but was ineffective against bronchial hyperresponsiveness [80,83]. This latter finding is consistent with the inability of theophylline to modulate allergen-induced bronchial hyperresponsiveness in asthmatics [81,84]. The inhibitory effect of theophylline against the late asthmatic response in asthma may be a consequence of a restoration of T suppressor cell function since it has long been recognized that theophylline can increase T suppressor

cell function [85-88] and impair graft rejection *in vitro* [88] and *in vivo* [89]. Individuals who do not develop a late asthmatic response have been shown to recruit a greater proportion of CD8+ (suppressor) than CD4+ (helper) T lymphocytes in BAL fluid [90].

Recent clinical studies have confirmed the anti-inflammatory properties of theophylline. In two randomized, placebo-controlled studies [84,91], the effect of theophylline or placebo was investigated on various inflammatory indices following once- and twice-daily treatment for 1 and 5 weeks, respectively. The late asthmatic response was reduced in those subjects treated with theophylline after 5 weeks [84], despite a mean plasma concentration of only 7.8 mg/ml. The lack of effect of theophylline on the acute response is presumably due to the low plasma levels in these subjects. Inhibition of the late asthmatic response therefore was unlikely to be due to functional antagonism of airway smooth muscle shortening or inhibition of mast cell degranulation [84]. Similarly, various surrogate markers of the nasal inflammatory response to allergen challenge including the late phase response and accumulation/activation of eosinophils was significantly attenuated in allergic rhinitis subjects following chronic treatment with theophylline [92], which is again consistent with the anti-inflammatory property of this drug.

Analysis of bronchial biopsies taken from mild asthmatics treated with low dose theophylline over 6 weeks revealed a significant reduction in EG2+ staining cells (activated eosinophils) and total number of eosinophils [93], which may be a consequence of the ability of theophylline to induce apoptosis of human eosinophils [94,95]. Similarly, a reduction in CD3+ T lymphocytes and expression of various activation markers on CD4+ T lymphocytes including HLA-DR and VLA-I was observed in BAL fluid [96]. Furthermore, a reduction in CD4+, CD8+ T-lymphocytes and IL-4 and IL-5 containing cells was observed in bronchial biopsies from asthmatics who were taking theophylline over a 6-week period [97] and a fall in circulating levels of Th2 cytokines, IL-4 and IL-5, was observed after a single low dose of theophylline [98].

In other studies, withdrawing theophylline from asthmatics who were taking glucocorticosteroids resulted in a significant deterioration of their disease [99,100], together with a concomitant rise in the number of CD4+, and CD8+ T lymphocytes in bronchial biopsies [100]. The mechanism of the inhibitory action of theophylline on lymphocytes in the airways may be a consequence of the ability of theophylline to inhibit lymphocyte chemotaxis [101] and/or induce apoptosis [95]. Furthermore, theophylline inhibits Th2 cytokine release from mononuclear cells and Th2 cell clones [68,102], although its inhibitory effect on cytokine synthesis is controversial [102,103].

Recently, two studies have demonstrated that in asthmatics who were poorly controlled on existing glucocorticosteroid therapy, a significant improvement in a number of clinical outcomes including, peak expiratory flow, FEV₁, symptom scores and reduced rescue medication was observed when patients were taking theophylline together with low dose glucocorticosteroid compared with high glucocorticosteroid treatment [104,105]. In both studies, the plasma levels of

theophylline measured were unlikely to be sufficient to induce bronchodilation (median 7.8 mg/ml [104] and mean 10.1 mg/ml [105]).

The molecular mechanism by which theophylline suppresses inflammation is the subject of much controversy and detailed hypotheses are beyond the scope of this article. Suffice to say that a number of mechanisms have been proposed which include adenosine A_{2B} receptor antagonism [106], alteration of intracellular calcium mobilization [107], stimulation of endogenous catecholamine release [108], prostaglandin antagonism [109] and PDE inhibition [110]. However, inhibition of PDE remains a popular theory to explain much of the clinical effects of theophylline, and although a recent study has shown its ability to promote apoptosis of human eosinophils may be unrelated to PDE inhibition [94], but this is not a universal finding [95]. The possibility that the anti-inflammatory activity of theophylline may be attributable to inhibition of PDE has led to many pharmaceutical companies pursuing selective PDE inhibitors for the treatment of asthma.

Clinical studies of PDE inhibitors in asthma and COPD

PDE inhibitors are currently being developed for the treatment of asthma, although side effects including emesis have halted the progression of some examples of this class of drug into the clinic. To date, there are a limited number of clinical studies investigating the efficacy of PDE inhibitors in the treatment of asthma. Inhalation of zardaverine produced a modest bronchodilator effect in patients with asthma and unacceptable side effects of nausea and emesis were reported in a significant number of patients [111], while oral administration of cilostazol (PDE3 inhibitor; Otsuka) caused bronchodilation and bronchoprotection against methacholine challenge in healthy subjects at the expense of mild to severe headache [112]. AH-21-132 (benzafentrine, a mixed PDE3/4 inhibitor; Novartis) has also been reported to have significant bronchodilator activity in normal volunteers [113]; the PDE4 inhibitor, ibudilast significantly improved baseline airways responsiveness to spasmogens by 2-fold after 6 months treatment [114] and MKS-492 (PDE3 inhibitor; Novartis) attenuated the early and late asthmatic response in atopic asthmatics [115].

Recently, the orally active PDE4 selective inhibitors, CDP-840 [36••] and roflumilast (Byk Gulden) [116], modestly attenuate the development of the late asthmatic response in mild asthmatics whilst having no effect on the acute response, with no significant side effects being reported in comparison with placebo. The ability of these novel selective PDE4 inhibitors to inhibit the late asthmatic response was not associated with bronchodilation, suggesting actions of this drug other than smooth muscle relaxation. Furthermore, the PDE4 inhibitor, RP-73401, also had no significant effect on allergen-induced bronchoconstriction in allergic asthmatic subjects [117], consistent with the suggestion that PDE3 rather than PDE4 may be the important isoenzyme regulating airway smooth muscle tone in asthmatic subjects. However, recent clinical studies with another orally active PDE4 inhibitor, SB-207499, have shown that this drug can attenuate bronchoconstriction following exercise in asthmatic subjects [118], an effect mimicked by 4 weeks of treatment with the selective PDE4 inhibitor, roflumilast [119].

More recently, SB-207499 administered to asthmatic subjects taking inhaled glucocorticosteroids or individuals with COPD [37••] demonstrated improvements in baseline lung function and was well tolerated with doses up to 15 mg bid. The mechanism of the beneficial action observed with SB-207499 is unlikely to be due to bronchodilation per se, since it has modest effects on airway smooth muscle function [120]. An explanation for its beneficial effects might include suppression of bronchial hyperresponsiveness secondary to a reduction of airway inflammation that would lead to improvements in lung function and/or reduction in afferent nerve activity and thereby reducing reflex bronchoconstriction.

Conclusion

It is therefore clear that encouraging signs are beginning to emerge concerning the development of novel PDE4 inhibitors which will not only assist in our understanding of the role of PDE4 subtypes in the regulation of cell function, but also offer the potential to find novel treatments for respiratory diseases [121].

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Anti-inflammatory

Anti-inflammatory

Cardiovascular

CNS

Oncological

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Anti-inflammatory

Anti-inflammatory

Cardiovascular

CPNS

Oncofertility



PDE4 inhibitors in COPD—a more selective approach to treatment

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Received 4 December 2003; accepted 11 December 2003

KEYWORDS

Phosphodiesterase 4 inhibitors;
Chronic obstructive pulmonary disease;
Cilomilast;
Anti-inflammatory;
Theophylline

Summary Chronic obstructive pulmonary disease (COPD) is a serious and mounting global public health problem. Although its pathogenesis is incompletely understood, chronic inflammation plays an important part and so new therapies with a novel anti-inflammatory mechanism of action may be of benefit in the treatment of COPD. Cilomilast and roflumilast are potent and selective phosphodiesterase (PDE)4 inhibitors, with an improved therapeutic index compared with the weak, non-selective PDE inhibitor, theophylline. Unlike theophylline, which is limited by poor efficacy and an unfavourable safety and tolerability profile, the selective PDE4 inhibitors are generally well tolerated, with demonstrated efficacy in improving lung function, decreasing the rate of exacerbations and improving quality of life, with proven anti-inflammatory effects in patients with COPD. Theophylline is a difficult drug to use clinically, requiring careful titration and routine plasma monitoring due to the risk of toxic side effects, such as cardiovascular and central nervous system adverse events, with dose adjustments required in many patients, including smokers, the elderly and some patients on concomitant medications. In contrast, the selective PDE4 inhibitors are convenient medications for both patient and physician alike. Hence these agents represent a therapeutic advance in the treatment of COPD, due to their novel mechanism of action and potent anti-inflammatory effects, coupled with a good safety and tolerability profile.

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Introduction

Globally, chronic obstructive pulmonary disease (COPD) is a serious and mounting public health problem. The prevalence of COPD, currently estimated at 600 million worldwide by the World Health Organization (WHO), may be underdiagnosed by as much as 50%.^{1–3} COPD was the sixth leading cause of death in 1990 according to the Global Burden of Disease (GBD) Study and is predicted to rise to third place by 2020.⁴ Moreover,

the GBD Study predicts that COPD will become the fifth highest ranking disease worldwide by 2020 in terms of disability-adjusted life years (DALYs), further reflecting the extent of the burden imposed by the condition.⁵

According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), a collaborative project of the US National Heart, Lung and Blood Institute and the World Health Organization, COPD fails to receive adequate attention from the healthcare community and government policy makers and, historically, information concerning its aetiology and prevalence has been sparse.^{6,7} As defined by the GOLD scientific committee, COPD is a disease state characterized by airflow limitation

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that is not fully reversible.^{6,7} Airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases, in particular, tobacco smoke.^{6,7} COPD appears to be a multi-component disease, characterized by airway inflammation, airway obstruction, structural remodelling of the airway wall and mucus hypersecretion, as well as systemic changes, including systemic inflammation, nutritional abnormalities and weight loss, and skeletal muscle dysfunction.⁶⁻⁸ Of note, these components may lead to acute exacerbations of COPD, defined by increased symptoms and worsening lung function, which have profound effects on both quality of life and healthcare costs.⁹⁻¹¹

Current pharmacological therapies for the treatment of COPD have limitations, particularly in relation to reducing the progression of the disease.^{1,12} Given the multi-component nature of COPD, multiple therapies will probably be required to treat it and one strategy may be to use current treatment options in combination.¹² However, since chronic inflammation is a critical underlying factor in the development of COPD, new therapies with a novel anti-inflammatory mechanism of action may provide additional benefits. One potential candidate is the selective phosphodiesterase (PDE)4 inhibitors, which have proven anti-inflammatory effects, coupled with an improved therapeutic ratio versus existing non-selective PDE inhibitors.^{13,14}

In this article, I describe the significant role that inflammation appears to play in the pathogenesis of COPD, the impact of PDE4 inhibitors in reducing inflammation via increased accumulation of the cyclic nucleotide, adenosine 3',5'-cyclic monophosphate (cAMP), and the potential advantages of second generation, selective PDE4 inhibitors (e.g. cilomilast and roflumilast) over first generation compounds (e.g. rolipram) and non-selective PDE inhibitors (e.g. theophylline) in the treatment of COPD.

Mechanism of chronic inflammation in COPD

Although the processes underlying COPD are not yet fully elucidated, chronic inflammation appears to have an important part (Fig. 1). Of note, substantial differences are apparent compared with the inflammatory mechanisms involved in the development of asthma.^{12,15} Inflammation plus structural alterations occurring in the small airways and lung parenchyma, such as fibrosis, smooth

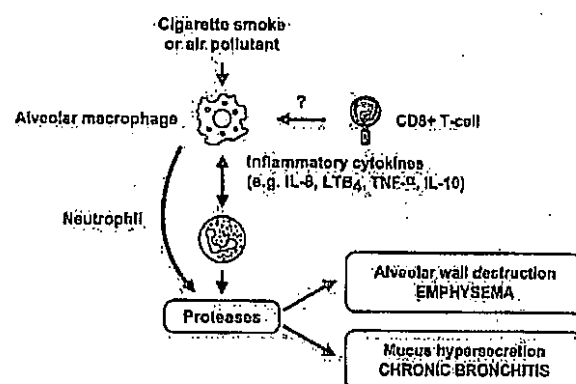


Figure 1 Pathogenesis of COPD, IL-8 = interleukin-8; LTB₄ = leukotriene B₄; TNF- α = tumor necrosis factor- α ; IL-10 = interleukin-10.

muscle hypertrophy, goblet cell metaplasia and lumen occlusion by mucus plugging, are major contributors to the airflow limitation and accelerated decline of forced expiratory volume in one second (FEV₁) observed in COPD.^{15,16} Key inflammatory cells implicated in COPD pathophysiology include neutrophils, macrophages and T cells (predominantly, CD8+ cells).^{15,16} In addition, structural cells, e.g. epithelial cells, fibroblasts and smooth muscle cells, participate via the release of a variety of mediators, including cytokines, chemokines and growth factors, such as leukotriene B₄ (LTB₄), interleukin (IL)-8 and tumour necrosis factor (TNF)- α .^{6,15,16} It has been proposed that an imbalance between pro- and anti-inflammatory cytokines may be involved in the process of neutrophil accumulation, for example, IL-8 and TNF- α levels are elevated.¹⁶ Together, airway inflammation and remodelling may also lead to degradation of the lung parenchyma, most likely mediated through release of proteolytic enzymes (such as elastase) from inflammatory cells, resulting in emphysema.^{15,16}

Degradation of cAMP by the PDEs (in particular, PDE4) has been hypothesized to be an important target to reduce the chronic inflammation, that is, characteristic of COPD. cAMP is a secondary messenger involved in signal transduction in a range of cellular processes, including cellular growth, sensory signalling, neuroplasty and transcription, as well as inflammation.¹⁷ Increases in cAMP levels result in activation of protein kinase A (PKA) and enhanced protein phosphorylation, which in turn leads to inhibitory effects on many inflammatory and immunomodulatory cells.¹⁸ These effects include relaxation of airway smooth muscle, inhibition of chemotaxis, abnormal release of inflammatory and cytotoxic mediators and inhibition of proliferation (as well as infiltration)

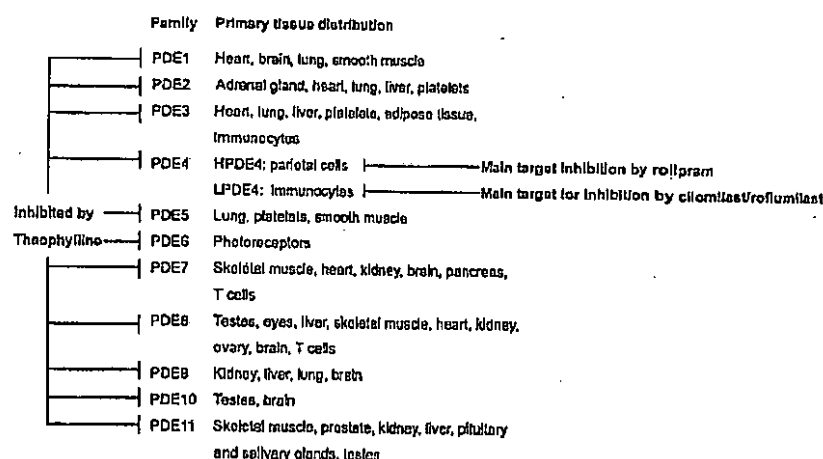


Figure 2 Tissue distribution of human PDEs, adapted with permission from Essayan.¹⁹

Cell	cAMP-PDEs
Basophils	3 and 4
B cells	Unknown
Eosinophils	4
Macrophages	3 and 4
Mast cells	3 and 4
Monocytes	4
Neutrophils	4
T cells	3, 4 and 7

Adapted with permission from Torphy.¹⁸

of inflammatory cells.^{18,19} Intracellular cAMP levels are regulated by a number of factors, notably by the PDEs, which catalyse the degradation of cAMP.^{17,18} The PDE superfamily consists of at least 11 isozymes, which vary in their distribution among different tissues/cell types (Fig. 2).¹⁹ The predominant isoenzyme in inflammatory and immunomodulatory cells, PDE4, is expressed in many airway cells involved in the pathogenesis of COPD, including neutrophils, macrophages, T cells and endothelial cells (Table 1).^{18,19} Hence, since PDE4 inhibition has the potential to produce significant anti-inflammatory and disease modifying effects by increasing accumulation of intracellular cAMP, PDE4 represents a promising molecular target for the treatment of COPD.^{14,20–22}

PDE4 inhibitors—mechanism of action and benefits over theophylline

In spite of their collective effects on PDE inhibition, there are a number of important differences

between the mechanisms of action of the non-selective PDE inhibitor, theophylline, and that of the selective PDE4 inhibitors (Table 2). Furthermore, second generation compounds have significant benefits over the first generation PDE4 inhibitors.

Theophylline

Theophylline, a broad spectrum PDE inhibitor, has been used to treat pulmonary diseases for over a century. It has established anti-inflammatory and immunomodulatory effects, possibly mediated by inhibition of the degradation of cAMP,^{23–25} although PDE inhibition may be negligible at therapeutically relevant concentrations of theophylline (see below).²⁶ However, although the mechanism of action of theophylline remains unclear, it appears to have a range of distinct features compared with the selective PDE4 inhibitors.²⁶

Firstly, theophylline is a weak and non-selective PDE inhibitor, which indiscriminately inhibits all PDEs in all tissues and organs of the body, including the gastrointestinal, cardiovascular and central nervous systems (Fig. 2).^{18,26,27} There is no evidence that theophylline has any selectivity for a particular isoenzyme, such as PDE4.²⁶ Non-selective PDE inhibition leads to elevation of cGMP, as well as cAMP, levels, resulting in the activation of both cAMP- and cGMP-dependent kinases (PKA and protein kinase G), linked with an increase in adverse events.^{26,28} Secondly, theophylline is a potent adenosine receptor antagonist at concentrations within the therapeutic range.^{26,29} This effect may be responsible for several of the serious side effects of theophylline, including seizures and cardiac arrhythmias.²⁶ Together, non-selective PDE inhibition and adenosine

Table 2. Comparison of cilomilast and theophylline.

	Theophylline	Cilomilast
Mechanism of action	Non-selective PDE inhibitor, leading to ↑ cAMP and ↓ cGMP ²⁶ PDE inhibition may be negligible at therapeutic concentrations ²⁶ Adenosine receptor antagonist ²⁶	Potent and selective PDE4 inhibitor, leading to ↑ cAMP only ²⁶ PDE4 inhibition at therapeutic concentrations ²⁶ No action on adenosine receptors ²⁶
Pharmacokinetics		
General	Non-linear pharmacokinetics, with significant inter-subject variability, including effects of smoking, age, concomitant medications—necessitate plasma monitoring ^{42,43,47,50,66}	Linear pharmacokinetics, unaffected by smoking—no requirement for plasma monitoring ^{49,53,67}
Absorption	Variable; depends on formulation ⁶⁷	Convenient oral tablet, dosed twice daily Rapid (T_{max} ~1–2 h), slower with food ^{49,53,67}
Bioavailability	Variable; depends on formulation ⁶⁷	100% Unaffected by food or co-administration of antacids ^{49,53}
Half-life	~7–9 h ⁶⁷	~7–8 h ^{49,53,67}
Volume of distribution	0.5 l/kg body weight; plasma-protein binding ~56% ⁵⁰	Low; plasma-protein binding ~99.5% ^{49,53}
Total plasma clearance	~0.4 l/h/kg; affected by genetic factors, environmental agents (including tobacco smoke), pathological conditions and concomitant drug treatments that affect hepatic metabolism ⁴⁷	Low (<2 l/h) ⁵³
Metabolism	~90% metabolized in liver by the CYP pathway, mainly by CYP1A2 (CYP2E1/CYP3A3/4 also contribute) ^{47,50}	Subject to negligible first-pass hepatic metabolism; extensively metabolized (main routes = decyclopentyl-acyl glucuronidation and 3-hydroxylation of the cyclopentyl-rine) ⁵³ Essentially no inhibition of CYP isoenzymes (CYP2C8) ⁵³
Drug interactions	Potential drug interactions include: propafenone, mexiletine, enoxacin, ciprofloxacin, cimetidine, propranolol, oral contraceptives, erythromycin, rifampicin, phenytoin, carbamazepine ^{47,50} phenobarbital, isoproterenol, tobacco smoke ^{47,50,52,61}	Low potential for drug interactions, and can be taken safely with many other medications that are commonly prescribed to patients with COPD ⁵³
Excretion	Only ~10% excreted via the kidneys in unchanged form ^{47,50}	Mainly excreted in urine (~90%) and faeces (6–7%); unchanged cilomilast accounts for less than 1% of administered dose ⁵³
Special populations	Dose adjustment may be required in smokers, the elderly, those with liver disease or patients taking concomitant medications; contraindicated in heart disease, seizure disorders and gastroesophageal reflux ^{46,47,50,52,54}	No dose adjustment required in smokers or the elderly ⁵³ ; use with caution in mild to moderate hepatic impairment and severe renal impairment; contraindicated in severe hepatic impairment ⁵³
Clinical efficacy		
	Modest dose-response effect ⁴²	Dose-dependent improvements in lung function, with FEV ₁ improvements maintained over 6 months ^{52,57}
	Only effective in subset of patients ⁴²	Effective in smokers and non-smokers ⁵²
	Slow onset of effect ⁴²	Decreases rate of exacerbations and improves quality of life ^{55,56}
		Demonstrated anti-inflammatory effects on CD8 ⁺ T-cells and CD68 ⁺ macrophages from bronchial biopsies ⁵⁹
Safety and tolerability		
	Serious cardiovascular and central nervous system side effects ⁴²	No cardiovascular/central nervous system side effects ⁵³
	10–15% of patients experience gastrointestinal side effects, insomnia or other minor side effects ^{42,51}	Generally well tolerated—mild to moderate GI effects may occur, but these are self-limiting and easily managed ⁵³

antagonism are likely to be responsible for many or all of the toxic side effects associated with theophylline use.^{26,27,29}

Of note, the degree of PDE inhibition may be negligible at concentrations of theophylline that are therapeutically relevant.²⁶ For example, in human lung extracts, theophylline at therapeutic concentrations has been shown to inhibit total PDE activity by only 5–10%.²⁶ There is growing evidence that theophylline works via other mechanisms than PDE inhibition and recent findings from asthma studies have indicated that its mode of action involves modification of histone acetylation, leading to changes in gene expression.^{26,30} However, theophylline's precise mechanism of action in COPD remains unknown.

First generation PDE4 inhibitors (e.g. roflupram)

Preclinical studies with first generation compounds such as roflupram that selectively inhibit PDE4 have demonstrated impressive activity in animal models of pulmonary inflammation.^{18,27,31} However, these agents were found to be associated with significant class—associated side effects, such as nausea and vomiting, and gastric acid secretion, caused by inhibition of PDE4 in the central nervous system and parietal glands, respectively.¹⁴ These findings necessitated the development of PDE4 inhibitors with an improved therapeutic ratio.

Second generation PDE4 inhibitors (e.g. cilomilast and roflumilast)

The most clinically advanced selective PDE4 inhibitors (cilomilast and roflumilast) have a superior side effect profile compared with theophylline and first generation compounds.¹⁴ These compounds were designed with the knowledge that PDE4 exists in two distinct conformations, high affinity roflupram-binding PDE4 (HPDE4; which predominates in the central nervous system and parietal glands) and low affinity roflupram-binding PDE4 (LPDE4; which predominates in immunocompetent cells).¹⁴ Unlike roflupram, which targets HPDE4, second generation compounds (such as cilomilast) primarily target LPDE4 (Fig. 2), resulting in an improved therapeutic index.¹⁴

Selective inhibition of PDE4 increases cAMP content in many inflammatory and immunomodulatory cells, leading to suppression of numerous aspects of the inflammatory response, as well as effects on smooth muscle and pulmonary nerves (Fig. 3). Indeed, second generation PDE4 inhibitors

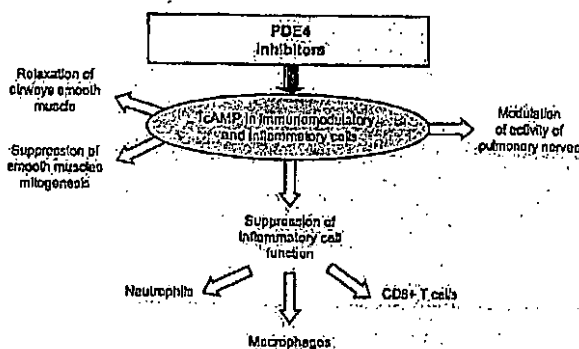


Figure 3 Mechanisms of PDE4 inhibition in COPD, adapted with permission from Torphy et al.¹⁴

have demonstrated pronounced anti-inflammatory effects in various animal models.^{18,21,22,32–34} Cilomilast/roflumilast inhibit the activity of cells that have been implicated in the pathogenesis of COPD, e.g. neutrophils,^{35,36} monocytes,³⁵ macrophages,³⁵ CD4T cells,³⁵ epithelial cells³⁷ and fibroblasts.^{38,39} Additionally, cilomilast has recently been shown to decrease levels of CD8 + T cells and CD68 + macrophages, thus demonstrating its potent anti-inflammatory effects.⁴⁰ Other effects of cilomilast and roflumilast include reduced chemotaxis, activation, degranulation and adherence of inflammatory cells, impact on key mechanisms involved in airway remodelling and modulation of the release of inflammatory mediators, such as TNF- α , IL-8, and GM-CSF.^{32,34,37–39,41} Significantly, cilomilast retains the anti-inflammatory actions of roflupram, but is substantially less likely to stimulate gastric acid secretion.²²

PDE4 inhibitors—pharmacokinetics and benefits over theophylline

The pharmacokinetics of the selective PDE4 inhibitors are markedly different from those of theophylline (for a detailed comparison of cilomilast versus theophylline, see Table 2). This has a number of implications in terms of the relative ease of use of these compounds and the extent of plasma monitoring required.

Theophylline is a difficult drug to use clinically, owing to significant inter- and intra-subject variability in pharmacokinetics.⁴² It has a narrow therapeutic margin and requires careful titration, with routine plasma monitoring, in order to reduce the risk of related adverse events.^{42,43} For example, potentially dangerous complications can

occur with theophylline when plasma levels rise above 20 µg/ml, e.g. cardiovascular events, including arrhythmias⁴⁴ and central nervous system events, including convulsions.⁴⁵ In particular, careful plasma monitoring and dose adjustments are required in smokers and elderly patients.^{3,46,47} This has important consequences for the cost of therapy. While theophylline is generally considered to be a low price medication, it has been estimated that total annual costs, including hospital admissions for toxic events and plasma monitoring, are higher for patients taking theophylline compared with other bronchodilators.⁴² In contrast to asthma, where recent evidence suggests that many of the beneficial effects of theophylline can be observed at low doses, there is evidence of a modest dose-response effect in patients with COPD,^{42,48} which is particularly relevant to the problems associated with its narrow therapeutic margin.

In contrast, the pharmacokinetics of cilomilast are unaffected by smoking.^{3,49} In the elderly, although there may be a small reduction in cilomilast clearance, this is not considered to be of clinical concern and does not necessitate dose adjustment.⁴⁹

Use of theophylline is also complicated by the range of potential drug interactions that may occur. Theophylline is metabolized by the cytochrome P450 (CYP) pathway, predominantly by CYP1A2, with a smaller contribution from CYP2E1 and CYP3A4. This leads to numerous potential drug interactions, including B₂ agonists, cimetidine, ciprofloxacin, lithium, prednisolone, oral contraceptives, rifampicin, digoxin, warfarin, propafenone, mexiletine, enoxacin, propranolol, erythromycin, phenytoin, carbamazepine, phenobarbital, isoproterenol and tobacco smoke.^{47,50-52} In contrast, cilomilast has a superior drug interactions profile compared with theophylline. Cilomilast does not inhibit any important CYP isoenzymes and therefore has a low potential for drug interactions.³ Although CYP2C8 has been implicated in cilomilast metabolism, this enzyme has few other substrates or inhibitors.³ Importantly, there is no evidence of drug interactions between cilomilast and warfarin, theophylline, digoxin, aluminium/magnesium hydroxide antacid, prednisolone or salbutamol.^{3,53} Furthermore, several pathological conditions affect theophylline metabolism and clearance, including congestive heart failure, cirrhosis, pulmonary oedema, cor pulmonale/COPD, obesity and systemic viral infection.⁵⁰ Theophylline is contraindicated in patients with heart disease, seizure disorders and gastroesophageal reflux.⁵⁴

Clinical data—benefits of PDE4 inhibitors over theophylline

Efficacy

Theophylline is the most frequently prescribed oral bronchodilator for the chronic maintenance treatment of chronic obstructive airway disorders.⁴² However, there is some debate as to the role of theophylline in the treatment of COPD compared with newer agents (Table 2). For example, only a modest dose-response effect has been demonstrated, with just small changes in FEV₁, forced vital capacity (FVC) and peak expiratory flow rate (PEFR) observed over the range of doses that induce theophylline concentrations of 5–10 µg/ml, 10–15 µg/ml and 15–20 µg/ml.⁴² It has been suggested that high doses of theophylline may be needed to induce a significant increase in FEV₁, but even when this is achieved, its bronchodilator action is often limited.⁴² In fact, the slow onset of action of theophylline coupled with difficulties in achieving stable plasma levels mean that most effects do not occur until 2–6 weeks.⁴² Of further concern, its effectiveness may be restricted to a subset of patients with COPD and it may be difficult to predict responders.⁴² Additionally, withdrawal of theophylline should be carried out with caution because of possible deterioration in lung function.⁴² Given these efficacy concerns, together with its toxicity issues, use of theophylline as monotherapy in COPD should be restricted to the rare cases where patients cannot adequately administer inhalers.⁴² Indeed, GOLD guidelines recommend that inhaled bronchodilators are preferable to theophylline, when available.^{6,7}

In contrast, the selective PDE4 inhibitors have demonstrated encouraging efficacy to date. According to published data, the most advanced PDE4 inhibitor in clinical development is cilomilast. This compound has been shown to improve both pulmonary function and symptoms of COPD in 424 patients with moderate-to-severe disease, producing significant improvements in FEV₁, FVC and PEFR versus placebo over 6 weeks.⁵⁵ In this study, cilomilast use was also associated with improvements in rescue bronchodilator use, exertional dyspnea, and resting and post-exercise arterial oxygen saturation (SaO₂).^{14,55} In addition, consistent improvements in patient quality of life approaching that defined as clinically relevant were observed compared with placebo.^{3,55} Furthermore, cilomilast significantly reduces the risk of exacerbations, both self-managed and those requiring physician intervention or hospitalization,

and provides sustained improvements in lung function over 6 months in patients with mild-to-moderate disease.^{56,57} Importantly, the improvements in lung function observed with cilomilast are independent of smoking status.⁵⁸ More recently, the anti-inflammatory effects of cilomilast observed in preclinical studies have been confirmed in a small, randomized, placebo-controlled trial of 59 patients with COPD.⁴⁰ After 12 weeks of cilomilast therapy, bronchial biopsies taken from treated patients indicated that cilomilast significantly reduces levels of inflammatory markers, i.e. CD8+ T cells and CD68+ macrophages.⁴⁰ These results represent the first demonstration by any agent of a reduction in airway tissue inflammatory cells characteristic of COPD.⁴⁰

Roflumilast has also shown encouraging efficacy in patients with COPD, with significant improvements observed in FEV₁ and PEF_R versus baseline,⁵⁹ although only limited published data are available at present.

Safety and tolerability

A major benefit of cilomilast and roflumilast is their superior safety and tolerability profile versus theophylline and the first generation PDE4 inhibitors (Table 2).

Theophylline is associated with serious cardiovascular and central nervous system side effects, even at therapeutic doses.^{42,45,60} These effects are generally attributed to non-selective inhibition of PDEs, as well as concomitant adenosine receptor antagonism,^{26,27,29} and include tachycardia and serious arrhythmias, focal and generalized seizures, and coma.^{45,60} Acute theophylline overdose is associated with metabolic and electrolyte abnormalities, including hypokalaemia, hyperglycaemia, leukocytosis and elevated serum catecholamine levels.⁴⁵ Moreover, approximately 10–15% of patients receiving theophylline will experience gastrointestinal adverse events, insomnia or other minor side effects.⁴² Although side effects can be reduced by ensuring that theophylline plasma concentrations do not enter the high therapeutic range, many physicians do not provide adequate monitoring of theophylline levels before or during long-term treatment.⁴² Despite the low price of theophylline, the overall cost may be greater than other bronchodilators owing to its toxic effects, including hospital admissions and blood level monitoring.⁴²

When the first generation PDE4 inhibitors were developed, it was envisaged that these compounds would have fewer side effects than theophylline,

due to their selectivity for PDE4.¹⁴ However, these agents were associated with a number of adverse events, including nausea, vomiting and gastric acid secretion, which limit their clinical use.¹⁴

In contrast, second generation PDE4 inhibitors have an improved therapeutic ratio.¹⁴ Cilomilast has proven safe and well tolerated at doses of up to 15 mg in both short- and long-term dosing trials, with a low incidence of adverse events.^{3,55} Although cilomilast does cause some gastrointestinal adverse events, most notably, nausea, this is generally mild-to-moderate and self-limiting.⁵⁵ Cilomilast can also be taken safely with many other medications that are commonly prescribed to patients with COPD. Similarly, preliminary findings indicate that roflumilast is also safe and well tolerated in patients with COPD,^{61–65} although only limited published data are available at present.

Conclusions

COPD is a serious chronic condition, which is increasingly being recognized as having a significant inflammatory component. New treatments are required that reduce inflammation and delay disease progression, and inhibition of PDE4 represents a promising mechanism to treat COPD, given the resulting effects on inflammation and associated underlying disease processes. Although our preconceptions of theophylline as an agent with PDE inhibitory activity draws us to compare theophylline with specific second generation PDE4 inhibitors, this may not be consistent with the current state of our scientific understanding. Theophylline and first generation PDE4 inhibitors (e.g. rolipram) are limited by poor efficacy and unfavourable safety and tolerability, resulting from their lack of selectivity. In contrast, second generation PDE4 inhibitors (e.g. cilomilast and roflumilast) are more selective and preliminary efficacy and safety/tolerability findings from large clinical trials, and using newer methods of measuring efficacy, are promising. These agents are likely to be of benefit in the treatment of COPD, particularly in combination with other agents, due to their novel mechanism of action and anti-inflammatory effects, and without the hazards and inconvenience of older agents posed to the prescribing physician.

Acknowledgements

This manuscript was supported by an educational grant from GlaxoSmithKline.

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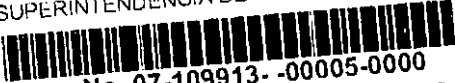
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