



Industria y Comercio
SUPERINTENDENCIA

PCT

DELEGATURA DE PROPIEDAD INDUSTRIAL

DIVISIÓN DE NUEVAS CREACIONES

07-39643

SOLICITUD DE PATENTE DE
INVENCION

TITULO: DERIVADOS DE XANTINA CON ACTIVIDAD DE RECEPTOR DE
HM74A.

SOLICITANTE: SMITHKLINE BEECHAM CORPORATION

DIRECCIÓN: ONE FRANKLIN PLAZA, P. O. BOX 7929, PHILADELPHIA,
PENNSYLVANIA 19101. ESTADOS UNIDOS DE AMERICA

A G L 31/522

APODERADO: J. IAN RAISBECK

BOGOTÁ 20 de Abril de 2006

P2007/000070

FM 22-05-2007

Cuota
de Retención

59



FORMULARIO ÚNICO DE SOLICITUD
PCT
ENTRADA EN FASE NACIONAL

SOLICITUD DE:

1

☒

Patente de Invención.

☐

Patente de Modelo de Utilidad.

☒

Capítulo I.

☐

Capítulo II.

2

SOLICITANTE
(71)

Nombre: SMITHKLINE BEECHAM COPORATION

Dirección: One Franklin Plaza, P.O Box 7929,
Philadelphia, PA 19101

Nacionalidad o Domicilio: Estadounidense

Lugar de Constitución: Estados Unidos de
América.

Teléfono: _____

Fax: _____

E-mail: _____

IDENTIFICACIÓN

C.C. ☐ NIT ☐

C.E. ☐ Otro ☐

Cual _____

Número _____

3

REPRESENTANTE
O APODERADO

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Dirección: World Trade Center Torre A,
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Teléfono: 601-7700 Fax: 601-7799

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IDENTIFICACIÓN

C.C. ☒ NIT ☐

C.E. ☐ Otro ☐

Cual _____

Número 79.376.996 Btá
TP 135.799

4

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Nacionalidad o Domicilio: BRITANICA.

5

PCT (86)

Solicitud Internacional No. PCT/EP2005/011376 Fecha Octubre 20, 2005

Publicación Internacional No. WO 2006/045565 Fecha Mayo 4, 2006

6

Título (54) DERIVADOS DE XANTINA CON ACTIVIDAD DE RECEPTOR DE
HM74A

7

Clasificación Internacional (51) C07D 473/04; C07D 743/06; A61K 31/522;
A61P 9/00

8

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GB

GB

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Octubre 22, 2004

Diciembre 10, 2004

(31) Número de Solicitud

0423568.5

0427079.9

9

Comprobante de pago No. 07- 29.614

Fecha 13 de Abril de 2007

10

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Poderes, si fuere el caso. **06-045.057**
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones.
- ☒ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).
- ☐ Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
- ☐ Traducción del examen preliminar internacional efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ De ser el caso, certificado de depósito del material biológico.
- ☐ Arte final 12 x 12.
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☐ De ser el caso, modificaciones efectuadas a la solicitud internacional.
- ☒ Otros. Búsqueda Internacional

12

Solicito la concesión de la patente,

NOMBRE: J. IAN RAISBECK

FIRMA:


C.C. 79.376.996 Btá T.P. 135.799

3

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 07 - 29,614
FECHA : ABRIL 13 DE 2007

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PASO	FECHA PASO	Vr. PASO
OLARTE RAISBEGR	CONSIGNACION	BANCO POPULAR	050-00110-6	56562732	12/04/2007	3,856,000.00

***** C O N C E P T O *****

CANT. RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1 50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	482,000.00
	TOTAL :	482,000.00

SOM: CUATROCIENTOS OCHENTA Y DOS MIL PESOS

RESPONSABLE : _____

**APOSTILLA EN PODER DE REPRESENTACIÓN DE
SMITHKLINE BEECHAM CORPORATION**

PODER DE REPRESENTACION

Bilingüe inglés- español otorgado en Brentford, Middlesex, Inglaterra el 13 de agosto de 2003 y firmado por Peter John Giddings, Apoderado y Funcionario autorizado.

Certificado de Notario De Pinna en español certificando que Peter John Giddings es apoderado de Smithkline Beecham Corporation, firmado por James Kerr Milligan.

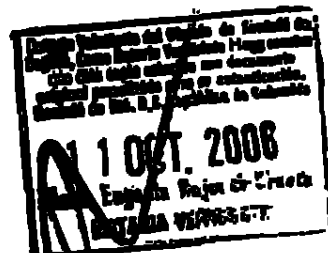
APOSTILLA

(Convención de la Haya del 5 de octubre de 1961)

1. País: Reino Unido de Gran Bretaña e Irlanda del Norte
- Este documento Público
2. ha sido firmado por James Kerr Milligan
3. Quien ejerce funciones de Notario público
4. Lleva el sello del Notario público

CERTIFICADO

5. En Londres
6. El 14 agosto 2003
7. Por el Secretario de Estado Principal de su Majestad para Asuntos exteriores y de la Mancomunidad
8. No. G228470
9. Gran Sello de la Oficina de Asuntos exteriores y de la Mancomunidad
10. Firma legible de J. Garbrah- Por el Secretario de Estado



Si el documento se usa en un país que no pertenece a la Convención de la Haya del 5 de octubre de 1961 debe pasar por la sección consular de la Misión que representa el país.

Es traducción fiel del inglés, con copia archivo para confrontación. Bogotá 21 agosto-03.

Marlen Neira C
Marlen Neira C

MARLEN NEIRA CASTRO
Traductor e Intérprete Oficial
Inglés - Español - Inglés
Resolución No. 2117 del 7 Oct 1987

OLARTE RAISBECK

OLARTE RAISBECK & FRIERI, LTDA.
LEGAL & PROFESSIONAL SERVICES

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Power of Attorney

The undersigned,

domiciled in

One Franklin Plaza, P O Box 7929, Philadelphia, PA 19101, United States of America
by means of these presents hereby grants to Carlos R. Olarte, J. Ian Ralsbeck and Ana Maria Frieri full and sufficient Power of Attorney to file, prosecute, obtain, defend and enforce any and all of the company's Patents, Utility Models and Industrial Designs in the Republic of Colombia, to which end it empowers them to take all steps necessary before any administrative or judicial authority of any class for the objects stated, to file petitions, appeals, replies and oppositions, pay taxes and annuities, request testimonies, receive documents and securities, abandon applications and collect refunds. Sufficient power is hereby granted to receive notifications on behalf of the Company, to confer powers on those who are to represent the company judicially and extrajudicially, to answer in court in the matter of all claims and demands that may be presented in connection with the objects stated, giving them likewise power to substitute these presents and revoke such substitutions.

El suscrito,

domiciliado en

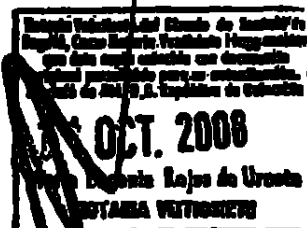
por medio del presente otorga a Carlos R. Olarte, J. Ian Ralsbeck y Ana Maria Frieri poder especial, amplio y suficiente para presentar, tramitar, obtener, defender y hacer respectar todas y cualquiera de Patentes, Modelos de Utilidad y Diseños Industriales de la compañía en la República de Colombia, a cuyo efecto los faculta para dar todos los pasos necesarios ante cualquier autoridad administrativa o judicial de cualquier jerarquía para cumplir con lo encomendado, elevar solicitudes, apelaciones, respuestas y oposiciones, pagar impuestos y anualidades, solicitar testimonios, recibir documentos y valores, desistir y percibir. Se concede poder bastante para recibir notificaciones a nombre de la compañía, otorgar poderes a quienes vayan a representar a la compañía judicial y extrajudicialmente, responder en juicio a todas las reclamaciones o demandas que por motivo de lo encomendado se presentaren, dándoles asimismo facultad para sustituir el presente y revocar dichas sustituciones.

Signature/Firma:


Peter John GIDDINGS as Attorney and Authorised Official

Date/Fecha: 13 August 2003

City/Country/Ciudad, País: Brentford, Middlesex, England



Yo, el infrascrito, **JAMES KERR MILLIGAN**, Notario Público de la Ciudad de Londres, Inglaterra, por la Autoridad Real debidamente admitido y juramentado,

CERTIFICO:

QUE es auténtica la firma "P. Giddings" suscrita al pie del adjunto Poder, por ser del puño y letra del Señor Don **PETER JOHN GIDDINGS**, cuya identidad me consta, Apoderado de la corporación denominada "**SMITHKLINE BEECHAM CORPORATION**", corporación legalmente constituida y existente de acuerdo con las leyes del Estado de Pensilvania, Estados Unidos de América, con domicilio social en 1, Franklin Plaza, Philadelphia, Pensilvania 19101, Estados Unidos de América;

QUE el citado Señor Don **PETER JOHN GIDDINGS** está debida y suficientemente autorizado para firmar documentos de esta clase, en virtud del Poder otorgado en su favor por la referida Sociedad fechado el 11 de julio de 2002, copia del cual he tenido a la vista.

Y QUE los objetos para los cuales se otorgó dicho Poder se encuentran dentro de los objetos o actividades de la citada Sociedad, de acuerdo con la Escritura Constitutiva y los Estatutos Sociales, los cuales he tenido a la vista.

Y PARA QUE CONSTE donde y como convenga y fuere necesario expido el presente Certificado que firmo y sello en dicha Ciudad de Londres, el día de hoy trece de agosto del año dos mil tres.



JAMES KERR MILLIGAN
Notario Público de Londres, Inglaterra



APOSTILLE
(Hague Convention of 5 October 1961 / Convention de La Haye du 5 octobre 1961)

UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

1. Country: United Kingdom of Great Britain and Northern Ireland
Pays: Royaume-Uni de Grande-Bretagne et d'Irlande du Nord

This public document / Le présent acte public

2. Has been signed by James Kerr Milligan
a été signé par

3. Acting in the capacity of Notary Public
agissant en qualité de

4. Bears the seal/stamp of The Said Notary Public
est revêtu du sceau/timbre de

5. at London/à Londres
6. the/le 14 August 2003

7. by Her Majesty's Principal Secretary of State for Foreign and Commonwealth Affairs /
par le Secrétaire d'Etat Principal de Sa Majesté aux Affaires Etrangères et du Commonwealth.

8. Number/num No **G229470**

9. Stamp:
timbre:

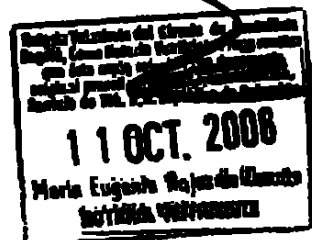
10. Signature: J. Garbrah

[Handwritten signature]



For the Secretary of State / Pour le Secrétaire d'Etat

If this document is to be used in a country which is not party to the Hague Convention of 5 October 1961,
it should be presented to the consular section of the mission representing that country.



(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
4 May 2006 (04.05.2006)

PCT

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WO 2006/045565 A1

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(30) Priority Data:
W23568.5 22 October 2004 (22.10.2004) GB
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(71) Applicant (for all designated States except US):
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(75) Inventors/Applicants (for US only): HATLEY, Richard,
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(74) Agent: BAKER, Suzanne, Jane; GlaxoSmithKline, Cor-
porate Intellectual Property (CN925.1), 980 Great West
Road, Brentford Middlesex TW8 9GS (GB).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AI, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
FR, GD, GE, GH, GM, GR, GU, ID, IL, IN, IS, JP, KE,
KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY,
MA, MD, MG, MK, MN, MW, MX, MY, NA, NG, NI, NO,
NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK,
SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.

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

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: XANTHINE DERIVATIVES WITH HM74A RECEPTOR ACTIVITY.

(57) Abstract: The present invention relates to therapeutically active compounds of formula (I) which are xanthine derivatives, processes for the manufacture of said derivatives, pharmaceutical formulations containing the active compounds and the use of the compounds in therapy, particularly in the treatment of diseases where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial.

WO 2006/045565 A1

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XANTHINE DERIVATIVES WITH HM74A RECEPTOR ACTIVITY

The present invention relates to therapeutically active compounds which are xanthine derivatives, processes for the manufacture of said derivatives, pharmaceutical formulations containing the active compounds and the use of the compounds in therapy, particularly in the treatment of diseases where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial.

Dyslipidaemia is a general term used to describe individuals with aberrant lipoprotein profiles. Clinically, the main classes of compounds used for the treatment of patients with dyslipidaemia, and therefore at risk of cardiovascular disease are the statins, fibrates, bile-acid binding resins and nicotinic acid. Nicotinic acid (Niacin, a B vitamin) has been used clinically for over 40 years in patients with various forms of dyslipidaemia. The primary mode of action of nicotinic acid is via inhibition of hormone-sensitive triglyceride lipase (HSL), which results in a lowering of plasma non-esterified fatty acids (NEFA) which in turn alters hepatic fat metabolism to reduce the output of LDL and VLDL (low and very low density lipoprotein). Reduced VLDL levels are thought to lower cholesterol ester transfer protein (CETP) activity to result in increased HDL (high density lipoprotein) levels which may be the cause of the observed cardiovascular benefits. Thus, nicotinic acid produces a very desirable alteration in lipoprotein profiles; reducing levels of VLDL and LDL whilst increasing HDL. Nicotinic acid has also been demonstrated to have disease modifying benefits, reducing the progression and increasing the regression of atherosclerotic lesions and reducing the number of cardiovascular events in several trials.

The observed inhibition of HSL by nicotinic acid treatment is mediated by a decrease in cellular cyclic adenosine monophosphate (cAMP) caused by the G-protein-mediated inhibition of adenylyl cyclase. Recently, the G-protein coupled receptors HM74 and HM74A have been identified as receptors for nicotinic acid (PCT patent application WO02/84298; Wise et. al. J Biol Chem., 2003, 278 (11), 9869-9874). The DNA sequence of human HM74A may be found in Genbank; accession number AY148884. Two further papers support this discovery, (Tunaru et. al. Nature Medicine, 2003, 9(3), 352-255 and Soga et. al. Biochem Biophys Res Commun., 2003, 303 (1) 384-389), however the nomenclature differs slightly. In the Tunaru paper what they term human HM74 is in fact HM74A and in the Soga paper HM74b is identical to HM74A. Cells transfected to express HM74A and/or HM74 gain the ability to elicit G_i G-protein mediated responses following exposure to nicotinic acid. In mice lacking the homologue of HM74A (m-PUMA-G) nicotinic acid fails to reduce plasma NEFA levels.

We now present a group of xanthine derivatives which are selective agonists of the nicotinic acid receptor HM74A and are thus of benefit in the treatment, prophylaxis and suppression of diseases where under-activation of this receptor either contributes to the disease or where activation of the receptor will be beneficial.

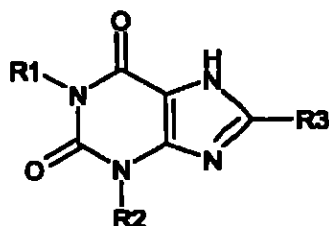
Summary of the Invention

The present invention provides therapeutically active xanthine derivatives and the use of these derivatives in therapy, particularly in the treatment of diseases where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial, in particular diseases of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia. As such, the compounds may also find favour as therapeutics for coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, as well as the cardiovascular indications associated with type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa and obesity. The compounds may also be of use in the treatment of inflammatory diseases or conditions, as set out further below.

Intermediates, formulations, methods and processes described herein form further aspects of the invention.

Detailed Description of the Invention

According to one aspect of this invention, we provide at least one chemical entity selected from compounds of formula (I)



(I)

and pharmaceutically acceptable derivatives thereof, wherein

R¹ represents a group selected from: hydrogen, C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, and -(alk)_m-X-(alk)_n-Y,

Wherein X represents A, A1, A2 or a direct link;

A represents a group selected from:

cycloalkylene, cycloalkenylene, aryl, heteroaryl, heterocyclyl, -CH₂-OC(O)-;

A1 represents a group selected from:

-CH₂-O-(CH₂)_n-aryl-O-, -CH₂-O-(CH₂)_n-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)-, -CH₂-(O)_p-(CH₂)_q-C(O)NR⁵-, -CH₂-N(R⁵)C(O)N(R⁵)-, -CH₂-C(O)N((CH₂)_wOH)-, -CH₂-NR⁵-S(O)₂-, CH₂-S(O)₂NR⁵-, -CH₂-C(O)O-, -O-, -NR⁵-, -S-;

A2 represents:

-CH(OH)-;

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5 When X is A, A1 or A2, Y represents a group selected from:

heteroaryl, heterocyclyl, aryl, cycloalkyl, cycloalkenyl, -O(CH₂)_n-aryl, -C(O)O-aryl, -CH(aryl)₂,
-CH(heteroaryl)₂, -C₁₋₆ haloalkyl, -C(O)R⁴, -NR⁵R⁷, -C(O)NR⁵R⁷, -NR⁵C(O)R⁷, -NR⁵C(O)OR⁷,
-C(O)(CH₂)_nOR⁴, halogen, cyano, -N(R⁵)C(O)OR⁷, -OC(O)NR⁵R⁸, -NR⁵C(O)R⁸, -OR⁸,
-OC(O)R⁴;

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When X is A1 and Y is selected from:

-O(CH₂)_n-aryl, -O-heteroaryl, -OR⁵, -OC(O)R⁵, -NH-aryl, -OC(O)NR⁵R⁸,
n is an integer selected from 2, 3, 4 and 5;

15 When X is A1 and Y is -CF₃, or when X is A2, n is an integer selected from 1, 2, 3, 4 and 5;

When X is a direct link, Y represents a group selected from:

-C(O)(CH₂)_nOR⁵, -C(O)-aryl, -C(O)-heteroaryl, -C(O)-heterocyclyl, -heteroaryl, -heterocyclyl,
-aryl, -cycloalkyl, -cycloalkenyl, -C₁₋₆ haloalkyl, -halo, -cyano, 3 or 4 ring fused system, -
20 CH(aryl)₂, -CH(heteroaryl)₂, -OR⁶, -NR⁶R⁷, -NCOOR⁶, -(O)_pC(O)NR⁶R⁸, -NR⁶C(O)R⁸, -OR⁶, -
(O)_pC(O)R⁴;

20

When Y incorporates a ring, that ring may be optionally substituted by one or more of:

C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, halogen, -NH₂, (CH₂)₄-NR⁵R⁷,
25 -(CH₂)_q-(O)_p-(CH₂)_q-N(R⁵)C(O)OR⁶, -(CH₂)_q-N(R⁵)C(O)R⁸, -(CH₂)_q-(O)_p-(CH₂)_q-C(O)NR⁵R⁸,
-(CH₂)_q-N(R⁵)C(O)N(R⁶)R⁸, -(CH₂)_q-C(O)N((CH₂)_mOH)R⁸, -(CH₂)_q-N(R⁵)-S(O)₂R⁸,
-CH₂-S(O)₂N(R⁵)R⁸, -C₁₋₆ haloalkyl, -OCF₃, -OCH(F)₂, -OCH₂F, -COOR⁶, -OR⁶, -(R⁶)_pCN,
-S(O)₂R⁸, -(CH₂)_nheteroaryl, -(CH₂)_nheterocyclyl, -(CH₂)_ncycloalkyl, -(CH₂)_ncycloalkenyl, -
(CH₂)_naryl;

30

R² is selected from: hydrogen; or C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, cycloalkyl,
cycloalkenyl, heterocyclyl, aryl, and heteroaryl, each of which may be optionally substituted
by one or more of: C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, cycloalkyl, heterocyclyl, aryl,
heteroaryl, C₁₋₆ haloalkyl, halogen, -CN, -OR⁴, -(CH₂)_nCOR⁴, -C(O)OR⁴, -OCOR⁴,

35

-(CH₂)_nNR⁵R⁸, -(NH)_pCONR⁵R⁸, -OCONR⁵R⁷, and -NHC(O)OR⁷;

R³ is selected from: halogenated C₁₋₆ alkyl;

R⁴ is selected from: hydrogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, -(CH₂)_n cycloalkyl,
40 -(CH₂)_n cycloalkenyl, -(CH₂)_n heterocyclyl, -(CH₂)_n aryl, and -(CH₂)_n heteroaryl;

R⁵ and R⁶ are selected from: hydrogen and C₁₋₄ alkyl;

R⁷ is selected from: hydrogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, -(CH₂)_n cycloalkyl,
45 -(CH₂)_n cycloalkenyl, -(CH₂)_n heterocyclyl, -(CH₂)_n aryl, and -(CH₂)_n heteroaryl;

R^5 is selected from C_{1-4} alkyl;

R^6 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ cycloalkenyl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ aryl, and $-(CH_2)_n$ heteroaryl, CN;

m represents an integer selected from: 0, 1, 2, 3, 4 and 5;

n represents an integer selected from: 0, 1, 2, 3, 4 and 5;

p represents an integer selected from: 0 and 1;

q represents an integer selected from: 0, 1 and 2;

t represents an integer selected from: 1 and 2;

w represents an integer selected from: 2, 3 and 4.

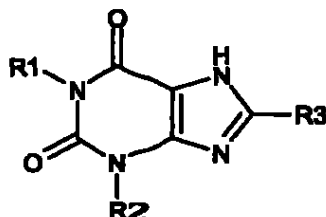
with the proviso that:

i) when R^1 represents hydrogen or C_{1-3} alkyl, R^2 is different to R^1 ;

ii) when R^1 represents ethyl and R^3 represents CF_3 , R^2 is other than $CH_2-CH=CH_2$; and

iii) when R^1 represents Methyl and R^3 represents CF_3 , R^2 is other than t -butyl.

In one embodiment of this invention, we provide at least one chemical entity selected from compounds of formula (I)



(I)

and pharmaceutically acceptable derivatives thereof, wherein

R^1 represents hydrogen or a group selected from: C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ aryl and $-(CH_2)_n$ heteroaryl, each of which may be optionally substituted by one or more of: $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ aryl, $-(CH_2)_n$ heteroaryl, C_{1-6} haloalkyl, halo, cyano, $-OR^4$, $-(CH_2)_nCOR^4$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^7$, $-(NH)_mCONR^5R^7$, $-OCONR^5R^7$, and $-NHCO_2R^4$;

R^2 is selected from: C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ aryl and $-(CH_2)_n$ heteroaryl, each of which may be optionally substituted by one or more of: cycloalkyl, heterocyclyl, aryl, heteroaryl, C_{1-6} haloalkyl, halo, cyano, $-OR^4$, $-(CH_2)_nCOR^4$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^7$, $-(NH)_mCONR^5R^7$,

$-\text{CONR}^5\text{R}^7$, and $-\text{NHCO}_2\text{R}^7$;

R^3 is selected from: halogenated C_{1-6} alkyl;

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5 R^4 is selected from: hydrogen, C_{1-6} alkyl C_{2-6} alkenyl, C_{2-6} alkynyl, $-(\text{CH}_2)_n\text{cycloalkyl}$, $-(\text{CH}_2)_n\text{heterocyclyl}$, $-(\text{CH}_2)_n\text{aryl}$, and $-(\text{CH}_2)_n\text{heteroaryl}$;

R^5 and R^6 are independently selected from: hydrogen and C_{1-4} alkyl;

10 R^7 is selected from: hydrogen, C_{1-6} alkyl C_{2-6} alkenyl, C_{2-6} alkynyl, $-(\text{CH}_2)_p\text{cycloalkyl}$, $-(\text{CH}_2)_p\text{heterocyclyl}$, $-(\text{CH}_2)_p\text{aryl}$, and $-(\text{CH}_2)_p\text{heteroaryl}$;

n represents an integer selected from: 0, 1, 2, 3 and 4;

15 m represents an integer selected from: 0 and 1;

p represents an integer selected from: 1 and 2.

with the proviso that:

- 20 i) when R^1 represents H or C_{1-3} alkyl, R^2 is different to R^1 ;
- ii) when R^1 represents ethyl and R^3 represents CF_3 , R^2 is other than $\text{CH}_2\text{-CH=CH}_2$; and
- 25 iii) when R^1 represents Methyl and R^3 represents CF_3 , R^2 is other than i-butyl.

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

30

As used herein, the terms "halogen" or "halo" refer to fluorine, chlorine, bromine and iodine.

35 As used herein, the term "alkyl" (when used as a group or as part of a group) refers to a straight or branched hydrocarbon chain unless specified otherwise, containing the specified number of carbon atoms. For example, $\text{C}_3\text{-C}_{10}\text{alkyl}$ means a straight or branched hydrocarbon chain containing at least 3 and at most 10 carbon atoms. Examples of alkyl as used herein include, but are not limited to methyl (Me), ethyl (Et), n-propyl and i-propyl.

40 As used herein, the term "alkenyl" refers to a straight or branched hydrocarbon chain containing the specified number of carbon atoms which contains one or more double bonds. Suitable examples include but are not limited to ethenyl, 2-propenyl, 3-butenyl, 2-butenyl, 2-pentenyl, 3-pentenyl, 3-methyl-2-butenyl, 3-hexenyl and the like.

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As used herein, the term "alkynyl" refers to a straight or branched hydrocarbon chain containing the specified number of carbon atoms which contains one or more triple bonds. Suitable examples include but are not limited to acetylenyl, propynyl, 1-butylnyl, 1-pentylnyl, 3-methyl-1-butylnyl and the like.

The term 'C₁₋₈ haloalkyl' as used herein refers to a C₁₋₈ alkyl group as defined herein wherein at least one hydrogen atom is replaced with halogen. Examples of such groups include fluoroethyl, trifluoromethyl or trifluoroethyl and the like.

As used herein, the term "cycloalkyl" refers to a hydrocarbon ring, containing between 3 and 6 carbon atoms, comprising no heteroatoms or conjugated double bonds. Examples of cycloalkyl as used herein include, but are not limited to cyclopropyl and cyclohexyl.

The term 'cycloalkylene' as used herein refers to a saturated monocyclic hydrocarbon ring of 3 to 8 carbon linker groups. Examples of such groups include cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, cycloheptylene or cyclooctylene and the like.

The term 'cycloalkenyl' as used herein refers to an unsaturated non-aromatic monocyclic hydrocarbon ring of 3 to 8 carbon atoms containing one or more carbon-carbon double bonds. Examples of such groups include cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl or cyclooctenyl and the like.

The term 'cycloalkenylene' as used herein refers to an unsaturated non-aromatic monocyclic hydrocarbon ring of 3 to 8 carbon linker groups containing one or more carbon-carbon double bonds. Examples of such groups include cyclopropenylene, cyclobutenylene, cyclopentenylene, cyclohexenylene, cycloheptenylene or cyclooctenylene and the like.

As used herein, the term "aryl" refers to a 5 or 6 membered, monocyclic aromatic group, or a fused 8-10 membered bicyclic aromatic group with at least one ring having a conjugated pi-electron system, containing up to two conjugated or fused ring systems. "Aryl" includes carbocyclic aryl and biaryl groups. Suitable examples include but are not limited to phenyl, naphthyl and the like.

As used herein, the term "heteroaryl" refers to a 5 or 6 membered, monocyclic aromatic group or a fused 8-10 membered bicyclic, aromatic group containing 1 to 3 heteroatoms independently selected from oxygen, nitrogen and sulphur with at least one ring having a conjugated pi-electron system, containing up to two conjugated or fused ring systems. Suitable examples of such monocyclic aromatic rings include but are not limited to thienyl, furyl, pyrrolyl, triazolyl, imidazolyl, oxazolyl, thiazolyl, oxadiazolyl, isothiazolyl, isoxazolyl, thiadiazolyl, pyrazolyl, pyrimidyl, pyridazinyl, pyrazinyl and pyridyl. Suitable examples of such fused aromatic rings include but are not limited to benzofused aromatic rings such as quinolinyl, isoquinolinyl, quinoxalinyl, quinoxaliny, cinnolinyl, naphthyridinyl, indolyl,

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indazolyl, pyrrolopyridinyl, benzofuranyl, benzothienyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzothiazolyl, benzisothiazolyl, benzoxadiazolyl, benzothiadiazolyl and the like.

5 As used herein, the term "heterocyclyl" refers to a 5 or 6 membered, saturated cyclic hydrocarbon group, containing 1 to 3 heteroatoms independently selected from oxygen, nitrogen and sulphur. Suitable examples include but are not limited to pyrrolidinyl, morpholinyl, imidazolidinyl and piperazinyl.

10 The term '3 or 4 ring fused system' as used herein refers to a fused 12-18 membered tricyclic or tetracyclic ring which contains 1 to 4 heteroatoms of N and wherein at least one ring is aromatic. There may be one or more optional oxo substituents on the ring carbon atoms. Examples of such fused aromatic rings include carbazolyl, acenaphthyl, naphthotriazolyl and the like.

15 As used herein, where a group is referred to as being "substituted" by another group or having "one or more substituents" unless a particular position for such a substitution is specified it is to be understood that a substitution may be present at any position in the group.

20 As used herein, the term "pharmaceutically acceptable derivative" refers to any pharmaceutically acceptable derivative of a compound of the present invention, for example salts, solvates or esters which, upon administration to a mammal, such as a human, are capable of providing (directly or indirectly) a compound of formula (I) or an active metabolite
25 or residue thereof. Such derivatives are clear to those skilled in the art, without undue experimentation, and with reference to the teaching of Burger's Medicinal Chemistry And Drug Discovery, 5th Edition, Vol 1: Principles And Practice, which is incorporated herein by reference.

30 It will be appreciated by those skilled in the art that the compounds of the invention may be modified to provide pharmaceutically acceptable derivatives thereof at any of the functional groups in the compounds, and that the compounds of the invention may be so modified at more than one position.

35 In one embodiment, "pharmaceutically acceptable derivative" refers to salts or solvates.

As used herein, the term "pharmaceutically acceptable" used in relation to an ingredient (active ingredient or excipient) which may be included in a pharmaceutical formulation for administration to a patient, refers to that ingredient being acceptable in the sense of being
40 compatible with any other ingredients present in the pharmaceutical formulation and not being deleterious to the recipient thereof.

15

As used herein, the term "solvate" refers to a complex of variable stoichiometry formed by a solute (In this invention, a compound of formula (I), a salt thereof or a pharmaceutically acceptable derivative thereof) and a solvent. Such solvents for the purposes of the present invention may not interfere with the biological activity of the solute. The solvent used may be a pharmaceutically acceptable solvent. Examples of suitable pharmaceutically acceptable solvents include water, ethanol and acetic acid. An example of a solvent that may be used is water, in which case the solvate may be referred to as a hydrate of the solute in question.

It will be appreciated that, for pharmaceutical use, the "salt or solvate" referred to above will be a pharmaceutically acceptable salt or solvate. However, other salts or solvates may find use, for example, in the preparation of a compound of formula (I) or in the preparation of a pharmaceutically acceptable salt or solvate thereof.

Pharmaceutically acceptable salts include those described by Berge, Bighley and Monkhouse, *J. Pharm. Sci.*, 1977, 66, 1-19. Suitable pharmaceutically acceptable salts include alkali metal salts formed from the addition of alkali metal bases such as alkali metal hydroxides. Examples of suitable alkali metal salts are sodium salt or potassium salt. Other suitable pharmaceutically acceptable salts include alkaline earth metal salts such as calcium salt or magnesium salt, ammonium salts; or salts with organic bases such as ethanolamine, triethanolamine, ethylene diamine, triethylamine, choline and meglumine; or salts with amino acids such as arginine, lysine and histidine.

The compounds are of use in the treatment of diseases where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial, in particular diseases of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia. As such, the compounds may also find favour as therapeutics for coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, as well as the cardiovascular indications associated with type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity. As such the compounds of the present invention may find use as agonists or partial agonists of HM74A.

Compounds of the invention are of potential therapeutic benefit in the treatment and amelioration of the symptoms of many diseases of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity. As such, the compounds may also find favour as therapeutics for coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke.

Furthermore, it is also believed that the HM74 and HM74A receptors are involved in inflammation. Inflammation represents a group of vascular, cellular and neurological responses to trauma. Inflammation can be characterised as the movement of inflammatory cells such as monocytes, neutrophils and granulocytes into the tissues. This is usually associated with reduced endothelial barrier function and oedema into the tissues. Inflammation with regards to disease typically is referred to as chronic inflammation and can last up to a lifetime. Such chronic inflammation may manifest itself through disease symptoms. The aim of anti-inflammatory therapy is therefore to reduce this chronic inflammation and allow for the physiological process of healing and tissue repair to progress.

Examples of inflammatory diseases or conditions for which the compounds of the present invention may demonstrate utility include those of the joint, particularly arthritis (e.g. rheumatoid arthritis, osteoarthritis, prosthetic joint failure), or the gastrointestinal tract (e.g. ulcerative colitis, Crohn's disease, and other inflammatory bowel and gastrointestinal diseases, gastritis and mucosal inflammation resulting from infection, the enteropathy provoked by non-steroidal anti-inflammatory drugs), of the lung (e.g. adult respiratory distress syndrome, asthma, cystic fibrosis, or chronic obstructive pulmonary disease), of the heart (e.g. myocarditis), of nervous tissue (e.g. multiple sclerosis), of the pancreas, (e.g. inflammation associated with diabetes mellitus and complications thereof, of the kidney (e.g. glomerulonephritis), of the skin (e.g. dermatitis, psoriasis, eczema, urticaria, burn injury), of the eye (e.g. glaucoma) as well as of transplanted organs (e.g. rejection) and multi-organ diseases (e.g. systemic lupus erythematosus, sepsis) and inflammatory sequelae of viral or bacterial infections and inflammatory conditions associated with atherosclerosis and following hypoxic or ischaemic insults (with or without reperfusion), for example in the brain or in ischaemic heart disease.

It will be appreciated that references herein to treatment extend to prophylaxis, prevention of recurrence and suppression of symptoms as well as the treatment of established conditions.

Nicotinic acid has a significant side effect profile, possibly because it is dosed at high level (gram quantities daily). The most common side effect is an intense cutaneous flushing. In certain embodiments of the present invention the compounds may exhibit reduced side effects compared to nicotinic acid. HM74A has been identified as a high affinity receptor for nicotinic acid whilst HM74 is a lower affinity receptor. The compounds of the present invention may find use as selective HM74A agonists or partial agonists; in which case they will show greater affinity for HM74A than for HM74.

The potential for compounds of formula (I) to activate HM74A may be demonstrated, for example, using the following in vitro whole cell assays:

In-vitro testing

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For transient transfections, HEK293T cells (HEK293 cells stably expressing the SV40 large T-antigen) were maintained in DMEM containing 10% foetal calf serum and 2mM glutamine. Cells were seeded in 90mm culture dishes and grown to 60-80% confluence (18-24h) prior to transfection. Human HM74A (GenBank™ accession number AY148884) was subcloned in to a mammalian expression vector (pcDNA3; Invitrogen) and transfected using Lipofectamine reagent. For transfection, 9µg of DNA was mixed with 30µl Lipofectamine in 0.8ml of Opti-MEM (Life Technologies Inc.) and was incubated at room temperature for 30min prior to the addition of 1.8ml of Opti-MEM. Cells were exposed to the Lipofectamine/DNA mixture for 5h and 6ml of 20% (v/v) foetal calf serum in DMEM was then added. Cells were harvested 48h after transfection. Pertussis toxin treatment was carried out by supplementation into media at 50ngml⁻¹ for 16h. All transient transfection studies involved co-transfection of receptor together with the G₁₆ G protein, G_{o1}α.

For generation of stable cell lines the above method was used to transfect CHO-K1 cells seeded in six well dishes grown to 30% confluence. These cells were maintained in DMEM F-12 HAM media containing 10% foetal calf serum and 2mM glutamine. 48h post-transfection the media was supplemented with 400µg/ml Geneticin (G418, Gibco) for selection of antibiotic resistant cells. Clonal CHO-K1 cell lines stably expressing HM74A were confirmed by [³⁵S]-GTPγS binding measurements, following the addition of nicotinic acid.

P2 membrane preparation - Plasma membrane-containing P2 particulate fractions were prepared from cell pastes frozen at -80°C after harvest. All procedures were carried out at 4°C. Cell pellets were resuspended in 1 ml of 10mM Tris-HCl and 0.1mM EDTA, pH 7.5 (buffer A) and by homogenisation for 20s with a Ultra Turrax followed by passage (5 times) through a 25-gauge needle. Cell lysates were centrifuged at 1,000g for 10 min in a microcentrifuge to pellet the nuclei and unbroken cells and P2 particulate fractions were recovered by microcentrifugation at 16,000g for 30min. P2 particulate fractions were resuspended in buffer A and stored at -80°C until required.

[³⁵S]-GTPγS binding - assays were performed at room temperature in 384-well format based on methods described previously, (Wieland, T. and Jakobs, K.H. (1994) *Methods Enzymol.* 237, 3-13). Briefly, the dilution of standard or test compounds were prepared and added to a 384-well plate in a volume of 10µl. Membranes (HM74A or HM74) were diluted in assay buffer (20mM HEPES, 100mM NaCl, 10mM MgCl₂, pH7.4) supplemented with saponin (60µg/ml), Leadseeker WGA beads (Amersham; 250µg/well) and 10µM GDP, so that the 20µl volume added to each well contains 5µg of membranes. [³⁵S]-GTPγS (1170 Ci/mmol, Amersham) was diluted (1:1500) in assay buffer and 20µl added to each well. Following the addition of the radioligand, the plates were sealed, pulse spun and incubated for 4hours at room temperature. At the end of the incubation period the plates were read on a Leadseeker machine (VIEWLUX PLUS; Perkin-Elmer) to determine the levels of specific binding.

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These assays were refined by reducing the final assay volume to 10 μ l. For this 10 μ l assay a revised protocol was used. This involved the use of only 100 nl of standard or test compound per well of a 384-well plate and 1.5 μ g membrane and 100 μ g Leadseeker WGA beads. For the low volume protocol, membrane, beads and [35 S]-GTP γ S were mixed together and then 10 μ l of this mix were dispensed to each well. Incubation and plate read were identical for the 10 μ l and 50 μ l assays.

All exemplified compounds were tested in one or both of the [35 S]-GTP γ S binding assays described above (i.e. the 10 μ l and 50 μ l assays).

Data was analysed by curve fitting as carried out using a Four Parameter Logistical equation using the XC50 software package (max 2 points deleted from any one curve). Specific binding is expressed as pEC₅₀ and as % efficacy compared to the maximal response of nicotinic acid binding.

In-vivo testing

HM74A agonists can be tested in male Spague-Dawley rats (200-250g) which have been fasted for at least 12 hours prior to the study. The compounds are dosed intravenously at either 1 or 3mg/kg (5ml/kg) or by oral gavage at doses ranging from 1-30mg/kg (10ml/kg). Blood samples (0.3ml tail vein bleed) can be taken pre-dose and at three times post-dose (times ranging from 15 minutes to 6 hours post-dose). Each blood sample is transferred to a heparin tube (Becton Dickinson Microtainer, PST LH) and centrifuged (10,000g for 5 minutes) to produce a plasma sample. The plasma samples are assayed for levels of non-esterified fatty acids (NEFA) using a commercially available kit (Randox). Inhibition of plasma NEFA levels, relative to pre-dose levels, are used as a surrogate for HM74A agonist activity.

In order to determine whether HM74A compounds exhibit the flushing response associated with nicotinic acid they can be dosed to conscious guinea-pigs. Male Dunkin Hartley guinea pigs (300-600g; n=10-20 per group) are fasted for at least 12 hours, but not in excess of 24 hours prior to experimentation. Pre-study blood samples (0.5ml) are taken from each animal by cardiac puncture under recovery anaesthesia (isoflurane 3.5% with additional O₂ (1L/min)). Ear temperature measurements are taken by placing the left ear of each animal over an infra-red temperature probe. Measurements are taken at one minute intervals from 5 minutes pre-dose to 30 minutes post-dose. Temperature measurements are then taken at 15 minute intervals up to 2 hours post-dose. Animals receive test compounds by oral gavage (5ml/kg). Blood samples (0.5ml) are taken by cardiac puncture under terminal anaesthesia. Blood samples are taken from individual animals to provide data at 0.5, 1, 2, 3, and 4 hours post-dose. All blood samples are placed on a blood roller for 5 minutes then stored on ice until the end of the study. Following centrifugation (12000g for 5min) the

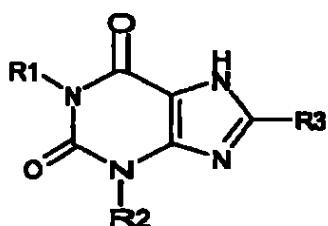
plasma is transferred into fresh tubes and stored at -20°C until assayed for NEFA concentrations.

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Compounds according to Formula (I) have been synthesised (see synthetic examples below) and tested in the [³⁵S]-GTPγS binding assays described above. All of the exemplified compounds have a pEC₅₀ of 4.3 or greater and an efficacy of 30% or greater (in relation to nicotinic acid).

Compounds of Formula (I) may find use in human or veterinary medicine, in particular as activators of HM74A, in the management of dyslipidaemia and hyperlipoproteinaemia.

According to another aspect of the invention, there is provided the use of at least one chemical entity selected from compounds of formula (II)



(II)

and pharmaceutically acceptable derivatives thereof, wherein

R¹ represents a group selected from: hydrogen, C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, and -(alk)_m-X-(alk)_n-Y,

Wherein X represents A, A1, A2 or a direct link;

A represents a group selected from:

cycloalkylene, cycloalkenylene, aryl, heteroaryl, heterocyclidyl, -CH₂-OC(O)-;

A1 represents a group selected from:

-CH₂-O-(CH₂)_a-aryl-O-, -CH₂-O-(CH₂)_w-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)-, -CH₂-(O)_p-(CH₂)_q-C(O)NR⁵-, -CH₂-N(R⁵)C(O)N(R⁵)-, -CH₂-N(R⁵)C(O)N((CH₂)_wOH)-, -CH₂-NR⁵-S(O)_z-, CH₂-S(O)_zNR⁵-, -CH₂-C(O)O-, -O-, -NR⁵-S-;

A2 represents:

-CH(OH)-;

When X is A, A1 or A2, Y represents a group selected from:

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heteroaryl, heterocyclyl, aryl, cycloalkyl, cycloalkenyl, $-(CH_2)_n$ -aryl, $-C(O)O$ -aryl, $-CH(aryl)_2$, $-CH(heteroaryl)_2$, $-C_{1-6}$ haloalkyl, $-C(O)R^4$, $-NR^5R^7$, $-C(O)NR^5R^7$, $-NR^5C(O)R^7$, $-NR^5C(O)OR^7$, $-C(O)(CH_2)_qOR^4$, halogen, cyano, $-N(R^5)C(O)OR^7$, $-OC(O)NR^5R^6$, $-NR^5C(O)R^6$, $-OR^6$, $-OC(O)R^4$;

5

When X is A1 and Y is selected from:

$-O(CH_2)_n$ -aryl, $-O$ -heteroaryl, $-OR^5$, $-OC(O)R^6$, $-NH$ -aryl, $-OC(O)NR^5R^6$,
n is an integer selected from 2, 3, 4 and 5;

10 When X is A1 and Y is $-CF_3$, or when X is A2, n is an integer selected from 1, 2, 3, 4 and 5;

When X is a direct link, Y represents a group selected from:

$-C(O)(CH_2)_qOR^5$, $-C(O)$ -aryl, $-C(O)$ -heteroaryl, $-C(O)$ -heterocyclyl, $-heteroaryl$, $-heterocyclyl$,
-aryl, $-cycloalkyl$, $-cycloalkenyl$, $-C_{1-6}$ haloalkyl, $-halo$, $-cyano$, 3 or 4 ring fused system,
15 $-CH(aryl)_2$, $-CH(heteroaryl)_2$, $-OR^5$, $-NR^5R^7$, $-NCOOR^5$, $-(O)_pC(O)NR^5R^6$, $-NR^5C(O)R^6$, $-OR^6$,
 $-(O)_pC(O)R^4$;

When Y incorporates a ring, that ring may be optionally substituted by one or more of:

C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, halogen, $-NH_2$, $(CH_2)_q-NR^5R^7$,
20 $-(CH_2)_q-(O)_p-(CH_2)_q-N(R^5)C(O)OR^6$, $-(CH_2)_q-N(R^5)C(O)R^6$, $-(CH_2)_q-(O)_p-(CH_2)_q-C(O)NR^5R^6$,
 $-(CH_2)_q-N(R^5)C(O)N(R^5)R^6$, $-(CH_2)_q-C(O)N((CH_2)_mOH)R^5$, $-(CH_2)_q-N(R^5)-S(O)_2R^6$,
 $-CH_2-S(O)_2N(R^5)R^6$, $-C_{1-6}$ haloalkyl, $-OCF_3$, $-OCH(F)_2$, $-OCH_2F$, $-COOR^5$, $-OR^5$, $-(R^5)_pCN$,
 $-S(O)_2R^6$, $-(CH_2)_n$ heteroaryl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ cycloalkenyl,
25 $-(CH_2)_n$ aryl;

R^2 is selected from: hydrogen; or C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, and heteroaryl, each of which may be optionally substituted by one or more of: C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, C_{1-6} haloalkyl, halogen, $-CN$, $-OR^4$, $-(CH_2)_nCOR^4$, $-C(O)OR^4$, $-OCOR^4$,

30 $-(CH_2)_nNR^5R^6$, $-(NH)_pCONR^5R^6$, $-CONR^5R^7$, and $-NHC(O)OR^7$;

R^3 is selected from: halogenated C_{1-6} alkyl;

R^4 is selected from: hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ cycloalkenyl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ aryl, and $-(CH_2)_n$ heteroaryl;

R^6 and R^6 are selected from: hydrogen and C_{1-4} alkyl;

R^7 is selected from: hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ cycloalkenyl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ aryl, and $-(CH_2)_n$ heteroaryl;

R^8 is selected from C_{1-4} alkyl;

R^9 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ cycloalkenyl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ aryl, and $-(CH_2)_n$ heteroaryl, CN;

m represents an integer selected from: 0, 1, 2, 3, 4 and 5;

n represents an integer selected from: 0, 1, 2, 3, 4 and 5;

5 p represents an integer selected from: 0 and 1;

q represents an integer selected from: 0, 1 and 2;

10 t represents an integer selected from: 1 and 2;

w represents an integer selected from: 2, 3 and 4;

15 in the manufacture of a medicament for the treatment of disorders of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia. In particular, the use is provided of a compound of Formula (II) in the manufacture of a medicament for the treatment of diabetic dyslipidaemia or mixed dyslipidaemia, heart failure, hypercholesterolaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity, coronary artery disease, thrombosis, angina, chronic renal failure, stroke and cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia.

20 In one embodiment of the invention, there is provided a compound of formula (I) or (II) or a pharmaceutically derivative thereof for use in the treatment of disorders of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia. In another embodiment, the use is provided of a compound of Formula (I) or (II) in the manufacture of a medicament for the treatment of diabetic dyslipidaemia or mixed dyslipidaemia, heart failure, hypercholesterolaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity, coronary artery disease, thrombosis, angina, chronic renal failure, stroke and cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia.

30 Thus, there is provided as a further aspect of the present invention a compound of formula (I) or (II) or a pharmaceutically acceptable derivative thereof, for use in human or veterinary medicine, particularly in the treatment of disorders of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity. As such, the compounds are also provided for use in the treatment of coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke.

40 There is provided as a further aspect of the present invention a compound of formula (I) or (II) or a pharmaceutically acceptable derivative thereof, for use in the manufacture of a medicament for the treatment of disorders of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and

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hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity. As such, the compounds are also provided for use in the treatment of coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke.

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In a further embodiment the present invention provides the use of a compound of formula (I) or (II) or a pharmaceutically acceptable derivative thereof, in the manufacture of a medicament for the treatment of inflammatory diseases or conditions of the joint, particularly arthritis (e.g. rheumatoid arthritis, osteoarthritis, prosthetic joint failure), or of the gastrointestinal tract (e.g. ulcerative colitis, Crohn's disease, and other inflammatory bowel and gastrointestinal diseases, gastritis and mucosal inflammation resulting from infection, the enteropathy provoked by non-steroidal anti-inflammatory drugs), of the lung (e.g. adult respiratory distress syndrome, asthma, cystic fibrosis, or chronic obstructive pulmonary disease); of the heart (e.g. myocarditis), of nervous tissue (e.g. multiple sclerosis), of the pancreas, (e.g. inflammation associated with diabetes mellitus and complications thereof, of the kidney (e.g. glomerulonephritis), of the skin (e.g. dermatitis, psoriasis, eczema, urticaria, burn injury), of the eye (e.g. glaucoma) as well as of transplanted organs (e.g. rejection) and multi-organ diseases (e.g. systemic lupus erythematosus, sepsis) and inflammatory sequelae of viral or bacterial infections and inflammatory conditions associated with atherosclerosis and following hypoxic or ischaemic insults (with or without reperfusion), for example in the brain or in ischaemic heart disease.

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In one embodiment, the compounds of formula (I) or (II) or pharmaceutically acceptable derivatives thereof are useful in the treatment and prevention of inflammation, diabetes and cardiovascular diseases or conditions including atherosclerosis, arteriosclerosis, hypertriglyceridemia, and mixed dyslipidaemia.

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In a further or alternative aspect there is provided a method for the treatment of a human or animal subject with a condition where under-activation of the HM74A receptor contributes to the condition or where activation of the receptor will be beneficial, which method comprises administering to said human or animal subject an effective amount of a compound of formula (I) or (II) or a pharmaceutically acceptable derivative thereof.

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In one embodiment the present invention provides a method for the treatment of disorders of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity, which method comprises administering to said human or animal subject an effective amount of a compound of formula (I) or (II) or a pharmaceutically acceptable derivative thereof. As such, these compounds may also find favour in methods for the treatment of coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, which methods comprise administering to said human or animal subject

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an effective amount of a compound of formula (I) or (II) or a pharmaceutically acceptable derivative thereof.

In one embodiment of formula (I) or (II), R^1 is selected from hydrogen C_{1-10} alkyl, and $-(alk)_m-X-(alk)_n-Y$.

In one embodiment of formula (I) or (II), X represents A or A1. In a further embodiment of formula (I) or (II), A is selected from heteroaryl, heterocyclyl, and A1 is selected from $CH_2-O-(CH_2)_w-N(R^5)C(O)O-$, for example $CH_2O(CH_2)_2NHC(O)O-$, $CH_2-N(R^5)C(O)O-$ for example $CH_2-NHC(O)O$, $CH_2-N(R^5)C(O)-$ for example, $CH_2-NHC(O)-$, $CH_2(O)_p-(CH_2)_qC(O)NR^5-$ for example $CH_2C(O)NCH_3$, $CH_2-N(R^5)C(O)N(R^5)-$ for example $CH_2-NHC(O)NCH_3$, $CH_2-C(O)N((CH_2)_wOH)-$ for example $CH_2-C(O)N((CH_2)_2OH)-$, $CH_2-NR^5-S(O)_2-$ for example $CH_2-NH-S(O)_2$, $CH_2-S(O)_2NR^5-$ for example $CH_2-S(O)_2NCH_3$, and $CH_2-C(O)O-$. In another embodiment of formula (I) or (II), X represents A and A represents a heteroaryl. In another embodiment of formula (I) or (II), A represents a heteroaryl comprising a nitrogen heteroatom, for example, triazolyl, furazanyl, oxadiazolyl, tetrazolyl, imidazolyl or pyrazolyl.

In a further embodiment of formula (I) or (II), X represents A, for example heteroaryl or heterocyclyl, or a direct link.

In one embodiment of formula (I) or (II), Y represents an optionally substituted group selected from: aryl, for example phenyl or naphthyl, heteroaryl, for example pyridinyl, thiazolyl, thienyl, benzofuranyl or indolyl, and O-aryl, for example O-phenyl.

In a further embodiment of formula (I) or (II), Y is substituted by one or more groups selected from OR^6 for example OH or OCH_3 , halogen, for example F or Cl, aryl, for example phenyl, C_{1-4} haloalkyl for example CF_3 or CH_2CF_3 , OCF_3 , $(R^6)_pCN$ for example CN, $(CH_2)_q-N(R^6)-S(O)_2R^6$ for example $NHS(O)_2CH_3$ and $S(O)_2R^6$ for example $S(O)_2CH_3$.

In one embodiment of formula (I) or (II), when X represents A or A1, and A represents cycloalkyl, cycloalkenyl, aryl, heteroaryl or heterocyclyl, and A1 represents $-CH_2-O-(CH_2)_w-N(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)-$, $-CH_2(O)_p-(CH_2)_qC(O)NR^5-$, $-CH_2-N(R^5)C(O)N(R^5)-$, or $-CH_2-C(O)N((CH_2)_wOH)-$, and Y represents a ring, for example when X represents oxadiazolyl, tetrazolyl or pyrazolyl and Y represents phenyl, pyridinyl, or thienyl, m is an integer selected from 3 and 4 and n is an integer selected from 0 and 1, for example m is 4 and n is 0, or m is 3 and n is 1;

In one embodiment of formula (I) or (II), R^1 is selected from: hydrogen; and C_{1-10} alkyl which may be optionally substituted by one or more of: cycloalkyl, heterocyclyl, aryl, heteroaryl, C_{1-4} haloalkyl, halo, cyano, $-OR^4$, $-(CH_2)_mCOR^5$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^7$, and $-(NH)_mCONR^5R^7$. In another embodiment of formula (I) or (II), R^1 is selected from: hydrogen; and C_{1-10} alkyl which may be optionally substituted by one or more of: halo, cyano, $-OR^4$, $-(CH_2)_mCOR^4$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^6$, and $-(NH)_mCONR^5R^6$.

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In a further embodiment of formula (I) or (II), R^1 is selected from: hydrogen and C_{1-8} alkyl optionally substituted with $-OH$, for example methyl, butyl or CH_2CH_2OH .

5 In one embodiment of formula (I) or (II), R^1 is selected from: hydrogen and C_{1-8} alkyl, for example methyl or butyl.

10 In one embodiment of formula (I) or (II), R^2 is selected from: C_{4-10} alkyl and C_{2-10} alkenyl, each of which may be optionally substituted by one or more of cycloalkyl, heterocyclyl, aryl, heteroaryl, C_{1-8} haloalkyl, halo, cyano, $-OR^4$, $-(CH_2)_nCOR^5$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^6$, and $-(NH)_mCONR^5R^6$. In another embodiment of formula (I) or (II), R^2 may represent C_{4-10} alkyl which may be optionally substituted by one or more of cycloalkyl, heterocyclyl, aryl, heteroaryl, C_{1-8} haloalkyl, halo, cyano, $-OR^4$, $-(CH_2)_nCOR^4$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^6$, and
15 $-(NH)_mCONR^5R^6$. In a further embodiment R^2 is selected from C_{4-8} alkyl, for example butyl or pentyl.

20 In one embodiment of formula (I) or (II), R^2 represents C_{1-10} alkyl which may be optionally substituted by one or more of: cycloalkyl, heterocyclyl, aryl, heteroaryl, C_{1-8} haloalkyl, halogen, $-CN$, $-OR^4$, $-(CH_2)_nCOR^5$, $-C(O)OR^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^6$, and $-(NH)_mCONR^5R^6$. In a further embodiment of formula (I) or (II), R^2 is selected from C_{3-8} alkyl, for example butyl or pentyl.

25 In one embodiment of formula (I) or (II), R^3 is selected from: fluorinated C_{1-8} alkyl. In another embodiment of formula (I) or (II), R^3 is selected from: fluorinated C_{1-4} alkyl, for example CF_3 , CHF_2 or CF_2CF_3 .

30 In one embodiment of formula (I) or (II), R^4 is selected from: hydrogen, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, $-(CH_2)_n$ cycloalkyl, $-(CH_2)_n$ heterocyclyl, $-(CH_2)_n$ aryl, and $-(CH_2)_n$ heteroaryl. In another embodiment of formula (I) or (II) R^4 is selected from: hydrogen and C_{1-4} alkyl.

35 In one embodiment of formula (I) or (II), R^1 is selected from: hydrogen and C_{1-8} alkyl optionally substituted with $-OH$, R^2 is selected from: C_{4-8} alkyl and R^3 is selected from: fluorinated C_{1-4} alkyl, with the proviso that when R^1 represents Methyl and R^3 represents CF_3 , R^2 cannot be t-butyl.

In one embodiment of formula (I) or (II), R^5 is hydrogen.

40 In one embodiment of formula (I) or (II), R^7 is selected from: hydrogen and C_{1-4} alkyl.

In further embodiment of formula (I) or (II,) it is provided that when X is A1, A1 is -O- and Y is a ring which is substituted by aryl or heteroaryl, then m is an integer selected from 3, 4 and 5.

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5 In one embodiment of formula (I) or (II), R¹ and R² are different.

It is to be understood that the present invention includes any combination of particular embodiments and covers all combinations of particular substituents described hereinabove for compounds of Formula (I) or (II).

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Particular compounds of the present invention include:

- 3-butyl-1-methyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione,
 1,3-dibutyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione,
 15 3-butyl-1-methyl-8-(pentafluoroethyl)-3,7-dihydro-1H-purine-2,6-dione,
 8-(difluoromethyl)-1-methyl-3-pentyl-3,7-dihydro-1H-purine-2,6-dione,
 3-pentyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione,
 1-(2-hydroxyethyl)-3-pentyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione,
 3-Butyl-1-{3-[3-(phenylmethyl)-1,2,4-oxadiazol-5-yl]propyl}-8-(trifluoromethyl)-3,7-dihydro-
 20 1H-purine-2,6-dione,
 4-[3-Butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]butanoic acid,
 3-Butyl-1-[4-(3-phenyl-1,2,4-oxadiazol-5-yl)butyl]-8-(trifluoromethyl)-3,7-dihydro-1H-purine-
 2,6-dione,
 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoic acid,
 25 3-Butyl-1-[4-[3-(2-fluorophenyl)-1,2,4-oxadiazol-5-yl]butyl]-8-(trifluoromethyl)-3,7-dihydro-1H-
 purine-2,6-dione,
 3-Butyl-1-[4-[3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl]butyl]-8-(trifluoromethyl)-3,7-dihydro-1H-
 purine-2,6-dione,
 3-Butyl-1-[4-[3-(2-pyridinyl)-1,2,4-oxadiazol-5-yl]butyl]-8-(trifluoromethyl)-3,7-dihydro-1H-
 30 purine-2,6-dione,
 3-Butyl-8-(trifluoromethyl)-1-(3-[3-[(2,4,6-trifluorophenyl)methyl]-1,2,4-oxadiazol-5-yl]propyl)-
 3,7-dihydro-1H-purine-2,6-dione,
 1-Methyl-3-pentyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione.

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The amount of a HM74A modulator which is required to achieve the desired biological effect will, of course, depend on a number of factors, for example, the mode of administration and the precise clinical condition of the recipient. In general, the daily dose will be in the range of 0.1mg - 1g/kg, typically 0.1 - 100mg/kg. An intravenous dose may, for example, be in the
 40 range of 0.01mg to 0.1g/kg, typically 0.01mg to 10mg/kg, which may conveniently be administered as an infusion of from 0.1µg to 1mg, per minute. Infusion fluids suitable for this purpose may contain, for example, from 0.01µg to 0.1mg, per millilitre. Unit doses may contain, for example, from 0.01µg to 1g of a HM74A modulator. Thus ampoules for injection

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may contain, for example, from 0.01µg to 0.1g and orally administrable unit dose formulations, such as tablets or capsules, may contain, for example, from 0.1mg to 1g. No toxicological effects are indicated/expected when a compound of the invention is administered in the above mentioned dosage range.

A compound of the present invention may be employed as the compound *per se* in the treatment of a disease where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial, an example of this is where a compound of the present invention is presented with an acceptable carrier in the form of a pharmaceutical formulation. The carrier must, of course, be acceptable in the sense of being compatible with the other ingredients of the formulation and must not be deleterious to the recipient. The carrier may be a solid or a liquid, or both, and may be formulated with the HM74A modulator as a unit-dose formulation, for example, a tablet, which may contain from 0.05% to 95% by weight of the HM74A modulator.

The formulations include those suitable for oral, rectal, topical, buccal (e.g. sub-lingual) and parenteral (e.g. subcutaneous, intramuscular, intradermal or intravenous) administration.

There is also provided according to the invention a process for preparation of such a pharmaceutical composition which comprises mixing the ingredients.

Formulations suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges or tablets, each containing a predetermined amount of a HM74A modulator; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. In general, the formulations are prepared by uniformly and intimately admixing the active HM74A modulator with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the product. For example, a tablet may be prepared by compressing or moulding a powder or granules of the HM74A modulator optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent and/or surface active/dispersing agent(s). Moulded tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid diluent.

Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch or polyvinyl pyrrolidone; fillers, for example, lactose, microcrystalline cellulose, sugar, maize-starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for example, potato starch, croscarmellose sodium or sodium starch glycolate; or wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions,

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emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxymethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan mono-oleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; or preservatives, for example, methyl or propyl p-hydroxybenzoates or sorbic acid. The preparations may also contain buffer salts, flavouring, colouring and/or sweetening agents (e.g. mannitol) as appropriate.

Formulations suitable for buccal (sub-lingual) administration include lozenges comprising a HM74A modulator in a flavoured base, usually sucrose and acacia or tragacanth, and pastilles comprising the HM74A modulator in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations for oral administration may be suitably formulated to give controlled/extended release of the active compound.

Formulations of the present invention suitable for parenteral administration conveniently comprise sterile aqueous preparations of an HM74A modulator, the formulation may be isotonic with the blood of the intended recipient. These preparations could be administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations may conveniently be prepared by admixing the HM74A modulator with water and rendering the resulting solution sterile and isotonic with the blood. Injectable compositions according to the invention will generally contain from 0.1 to 5% w/w of the HM74A modulator.

Thus, formulations of the present invention suitable for parenteral administration comprising a compound according to the invention may be formulated for parenteral administration by bolus injection or continuous infusion and may be presented in unit dose form, for instance as ampoules, vials, small volume infusions or pre-filled syringes, or in multi-dose containers with an added preservative. The compositions may take such forms as solutions, suspensions, or emulsions in aqueous or non-aqueous vehicles, and may contain formulatory agents such as anti-oxidants, buffers, antimicrobial agents and/or toxicity adjusting agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. The dry solid presentation may be prepared by filling a sterile powder aseptically into individual sterile containers or by filling a sterile solution aseptically into each container and freeze-drying.

Formulations suitable for rectal administration may be presented as unit-dose suppositories. These may be prepared by admixing a HM74A modulator with one or more conventional solid carriers, for example, cocoa butter or glycerides and then shaping the resulting mixture.

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Formulations suitable for topical application to the skin may take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include vaseline, lanolin, polyethylene glycols, alcohols, and combinations of two or more thereof.

5 The HM74A modulator is generally present at a concentration of from 0.1 to 15% w/w of the composition, for example, from 0.5 to 2%.

By topical administration as used herein, we include administration by insufflation and inhalation. Examples of various types of preparation for topical administration include
10 ointments, creams, lotions, powders, pessaries, sprays, aerosols, capsules or cartridges for use in an inhaler or insufflator or drops (e.g. eye or nose drops).

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents and/or solvents. Such bases may
15 thus, for example, include water and/or an oil such as liquid paraffin or a vegetable oil such as arachis oil or castor oil or a solvent such as a polyethylene glycol. Thickening agents which may be used include soft paraffin, aluminium stearate, cetostearyl alcohol, polyethylene glycols, microcrystalline wax and beeswax.

20 Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents or thickening agents.

Powders for external application may be formed with the aid of any suitable powder base, for
25 example, talc, lactose or starch. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilising agents or suspending agents.

Spray compositions may be formulated, for example, as aqueous solutions or suspensions
30 or as aerosols delivered from pressurised packs, with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, 1,1,1,2,3,3,3-heptafluoropropane, 1,1,1,2-tetrafluoroethane, carbon dioxide or other suitable gas.

Capsules and cartridges for use in an inhaler or insufflator, of for example gelatin, may be
35 formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

The pharmaceutical compositions according to the invention may also be used in
40 combination with other therapeutic agents, for example in combination with other classes of dyslipidaemic drugs (e.g. statins, fibrates, bile-acid binding resins or nicotinic acid).

The compounds of the instant invention may be used in combination with one or more other
therapeutic agents for example in combination with other classes of dyslipidaemic drugs e.g.

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3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) or fibrates or bile acid binding resins or nicotinic acid. The invention thus provides, in a further aspect, the use of such a combination in the treatment of diseases where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial and the use of a compound of formula (I) or (II), or a pharmaceutically acceptable salt, solvate or pharmaceutically acceptable derivative thereof in the manufacture of a medicament for the combination therapy of disorders of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa or obesity.

When the compounds of the present invention are used in combination with other therapeutic agents, the compounds may be administered either sequentially or simultaneously by any convenient route.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above optimally together with a pharmaceutically acceptable carrier or excipient comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When combined in the same formulation it will be appreciated that the two components must be stable and compatible with each other and the other components of the formulation and may be formulated for administration. When formulated separately they may be provided in any convenient formulation, conveniently in such a manner as are known for such compounds in the art.

When in combination with a second therapeutic agent active against the same disease, the dose of each component may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The invention thus provides, in a further aspect, a combination comprising a compound of formula (I) or a pharmaceutically acceptable derivative thereof together with another therapeutically active agent.

The combination referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable diluent, excipient or carrier thereof represent a further aspect of the invention. For example, a pharmaceutical formulation comprising a compound or pharmaceutically acceptable derivative thereof and one or more pharmaceutically acceptable diluents, excipients or carriers.

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5 General purification and analytical methods:

The mass spectra (MS) were recorded on a Fisons VG Platform mass spectrometer using electrospray positive ionisation [(ES+ve to give MH^+ and $M(NH_4)^+$ molecular ions] or electrospray negative ionisation [(ES-ve to give $(M-H)^-$ molecular ion] modes.

1H NMR spectra were recorded using a Bruker DPX 400MHz spectrometer using tetramethylsilane as the external standard.

BiotageTM chromatography refers to purification carried out using equipment sold by Dyax Corporation (either the Flash 40i or Flash 150i) and cartridges pre-packed with KPSII.

Mass directed autoprep refers to methods where the material was purified by high performance liquid chromatography on a HPLCABZ+ 5 μ m column (5cm x 10mm i.d.) with 0.1% HCO_2H in water and 95% MeCN, 5% water (0.5% HCO_2H) utilising the following gradient elution conditions: 0-1.0 minutes 5%B, 1.0-8.0 minutes 5 $\rightarrow$ 30%B, 8.0-8.9 minutes 30%B, 8.9-9.0 minutes 30 $\rightarrow$ 95%B, 9.0-9.9 minutes 95%B, 9.9-10 minutes 95 $\rightarrow$ 0%B at a flow rate of 8ml minutes⁻¹ (System 2). The Gilson 202-fraction collector was triggered by a VG Platform Mass Spectrometer on detecting the mass of interest.

Preparative h.p.l.c. refers to methods where the material was purified by high performance liquid chromatography on a HPLCABZ+ 5 μ m column (10cm x 21.2mm i.d.) with 0.1% HCO_2H in water (A) and MeCN (0.5% HCO_2H) (B) utilising the generic gradient elution conditions expressed as "x to y" gradient with a gradient system as follows: 0-1.45minutes x%B, 1.45-20 minutes x $\rightarrow$ y%B, 20-24 minutes y $\rightarrow$ 95%B, 24-30 minutes 95%B, 32-34 minutes 95 $\rightarrow$ x%B at a flow rate of 8ml minutes⁻¹. The Gilson 233 fraction collector was triggered by UV (254nm).

SPE (solid phase extraction) refers to the use of cartridges sold by International Sorbent Technology Ltd.

Strata Phenyl SPE refers to the use of cartridges sold by Phenomenex. The compound was loaded onto a cartridge previously conditioned with MeCN and equilibrated with 5% MeCN in water. The compound was eluted with 0.1% HCO_2H in water and MeCN (0.5% HCO_2H) in a suitable gradient on a Combiflash Optix 10.

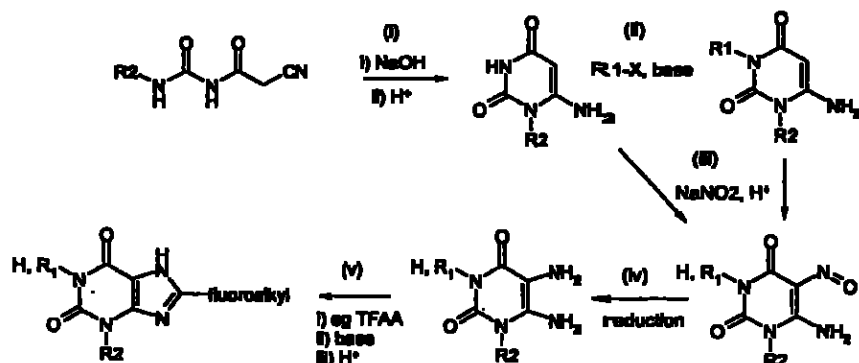
The compounds of the present invention and pharmaceutically derivatives thereof may be prepared by the methodology described hereinafter, constituting a further aspect of this invention.

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Process A:

A process according to the invention for preparing a compound of formula (I) or formula (II) in which R¹ is H or alkyl wherein R¹ and R² may be the same or different, comprises:



i) Pyrimidinedione formation

ii) Alkylation

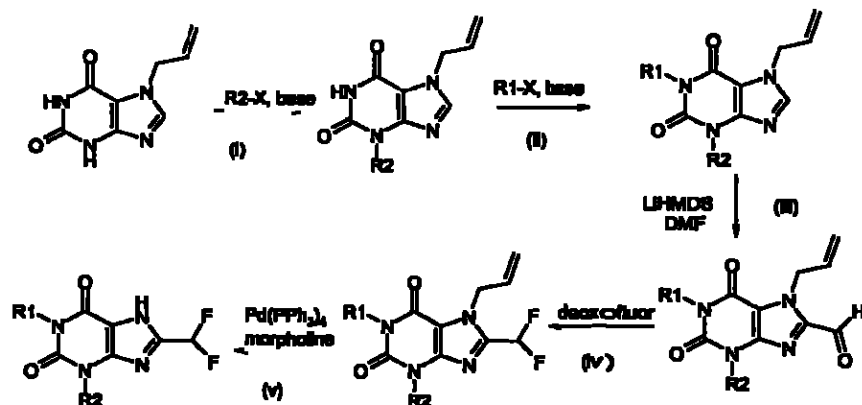
10 iii) Nitrosation

iv) Reduction

v) Xanthine formation

Process B:

A process according to the invention for preparing a compound of formula (I) or formula (II) in which R¹ and R² may be the same or different, comprises:



i) Selective alkylation at N3

ii) Alkylation at N1

iii) Formylation of C8

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- iv) Fluorination of carbonyl
- v) Palladium mediated deprotection

5 Where desired or necessary, as a final stage in any of the above synthetic processes, a resultant compound of formula (I) or (II) can be converted into a pharmaceutically acceptable salt form or vice versa or converting one salt form into another pharmaceutically acceptable salt form.

ABBREVIATIONS

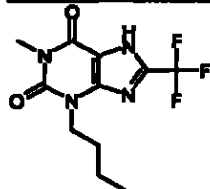
AcOH	Acetic acid
br	Broad (NMR)
CDI	Carbonyldiimidazole
d	Doublet (NMR)
DBAD	Di- <i>t</i> -butylazodicarboxylate
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMSO	Dimethylsulfoxide
DMF	N,N-Dimethylformamide
EtOAc	Ethyl acetate
EtOH	Ethanol
h	Hour(s)
IPA	Isopropyl alcohol
m	Multiplet (NMR)
MDAP	Mass directed autoprep
MeCN	Acetonitrile
MeOH	Methanol
min	Minute(s)
NCS	N-Chlorosuccinimide
PFPA	pentafluoropropionic anhydride
q	Quartet (NMR)
rt	Room temperature
RT	Retention time
s	Singlet (NMR)
SPE	Solid phase extraction cartridge
t	Triplet (NMR)
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic acid anhydride
THF	Tetrahydrofuran
DMEM	Dulbecco's Modified Eagle's Medium
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulphonic acid
LHMDS	Lithium hexamethyldisilamide

The following non-limiting examples illustrate the present invention:

Synthetic Examples

Example 1

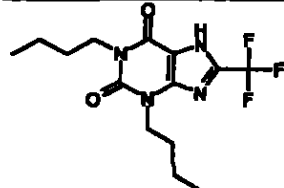
3-butyl-1-methyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione



A purple mix of 5,6-diamino-1-butyl-3-methyl-2,4(1H,3H)-pyrimidinedione (European Journal of Medicinal Chemistry (1990), 25(8), 653-8)(290mg) and TFAA (6ml) was heated at reflux for 1 hour then concentrated *in vacuo*. The resulting residue was treated with 2N NaOH (aq) (7ml) and the mixture heated at reflux for 30 minutes. After cooling to ambient temperature, the mixture was acidified to ca. pH4 with 3M HCl(aq). The precipitate was collected by filtration and washed with a solution of water:EtOH (2:1) then water:EtOH (1:1). The resulting beige solid was dried *in vacuo* (100mg, 25%). NMR; δ_H (400MHz, d^6 -DMSO) 0.90 (t, 3H, J=7.5Hz), 1.25-1.36 (m, 2H), 1.59-1.69 (m, 2H), 3.25 (s, 3H), 3.97 (t, 2H, J=7Hz), NH not observed to δ_H 15; m/z 291.2[MH⁺].

Example 2

1,3-dibutyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione

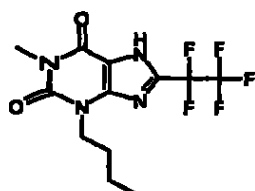


A purple mix of 5,6-diamino-1,3-dibutyl-2,4(1H,3H)-pyrimidinedione (Journal of Medicinal Chemistry (1991), 34(1), 466-9) (207mg) and TFAA (4ml) was heated at reflux for 1 hour then concentrated *in vacuo*. The resulting residue was treated with 2N NaOH(aq) (5ml) and the mixture heated at reflux for 30 minutes. After cooling to ambient temperature, the mixture was acidified to ca. pH4 with 3M HCl (aq). The precipitate was collected by filtration and purified by recrystallisation from a solution of water:EtOH (2:1). The pale brown/ orange solid was dried under vacuum at 50°C for 48 hours providing the title compound as a beige solid (35mg, 13%). δ_H (400MHz, d^6 -DMSO) 0.90 (t, 6H, J=7Hz), 1.26-1.34 (m, 4H), 1.48-1.68 (overlapping m, 4H), 3.89 (t, 2H, J=7Hz), 3.97 (t, 2H, J=7Hz), NH not observed to δ_H 15; m/z 333.2[MH⁺].

Example 3

3-butyl-1-methyl-8-(pentafluoroethyl)-3,7-dihydro-1H-purine-2,6-dione

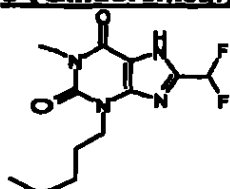
35



- A purple mix of 5,6-diamino-1-butyl-3-methyl-2,4(1H,3H)-pyrimidinedione (European Journal of Medicinal Chemistry (1990), 25(8), 653-8) (300mg, 1.41mmol) and pentafluoropropionic anhydride (PFPA) (6ml) was heated at 80°C for 1 hour then concentrated *in vacuo*. The resulting residue was treated with 2N NaOH (aq) (7ml) and the mixture heated at 90°C for 30 minutes. After cooling to room temperature the mixture was acidified to ca. pH4 with 3M HCl(aq). The precipitate was collected by filtration, washed with water and dried under vacuum affording the title compound as a pale green/brown fluffy solid (163mg, 34%). δ_H (400MHz, d^6 -DMSO) 0.89 (t, 3H, J=7.5Hz), 1.24-1.35 (m, 2H), 1.60-1.69 (m, 2H), 3.25 (s, 3H), 3.98 (t, 2H, J=7Hz), NH not observed to δ_H 15; m/z 358.4[MNH₄⁺].

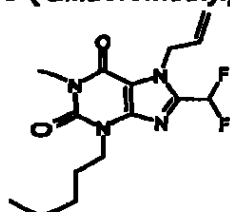
Example 4

8-(difluoromethyl)-1-methyl-3-pentyl-3,7-dihydro-1H-purine-2,6-dione



- A solution of 8-(difluoromethyl)-1-methyl-3-pentyl-7-(2-propen-1-yl)-3,7-dihydro-1H-purine-2,6-dione in anhydrous THF (3ml) and anhydrous DMSO (0.3ml) was degassed under a gentle vacuum and nitrogen introduced. This was repeated twice. Pd(PPh₃)₄ (74mg, 0.064mmol) was added and the mixture degassed once more. Morpholine (374μL, 4.3mmol) was added and left to stir at rt for 4 hours. The mixture was partitioned between 2M HCl(aq) and EtOAc. The organic layer was separated, washed with brine, dried (MgSO₄) and concentrated. The residue was taken up in MeOH and passed down and amino-propyl SPE column (5g), eluting with MeOH followed by 5% AcOH/MeOH. The product fraction was concentrated to afford the title compound as an off white solid (98mg, 80%). δ_H (400MHz, d^6 -DMSO) 0.85 (t, 3H, J=7Hz), 1.23-1.35 (m, 4H), 1.61-1.71 (m, 2H), 3.25 (s, 3H), 3.98 (t, 2H, J=7.5Hz), 7.12 (t, 1H, J=53Hz), 14.59 (br. s, 1H); m/z 287.2[MH⁺].

8-(difluoromethyl)-1-methyl-3-pentyl-7-(2-propen-1-yl)-3,7-dihydro-1H-purine-2,6-dione

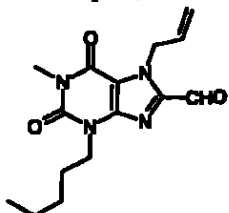


- 1-methyl-2,6-dioxo-3-pentyl-7-(2-propen-1-yl)-2,3,6,7-tetrahydro-1H-purine-8-carbaldehyde (133mg, 0.44mmol) was added to a pre-dried flask under nitrogen. Anhydrous DCM (3ml) was added followed by deoxofluor (161μL, 0.87mmol) and finally EtOH (1 drop, ca.

0.09mmol). The mixture was allowed to stir at rt. for 4 hours then partitioned between sat. $\text{NaHCO}_3(\text{aq})$ and DCM. The organic layer was separated, washed with brine, dried (MgSO_4) and concentrated to afford the title compound as a yellow oil (145mg). m/z 327.4 $[\text{MH}^+]$.

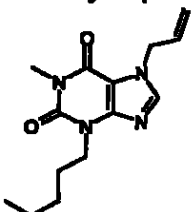
36

5 1-methyl-2,6-dioxo-3-pentyl-7-(2-propen-1-yl)-2,3,6,7-tetrahydro-1H-purine-8-carbaldehyde



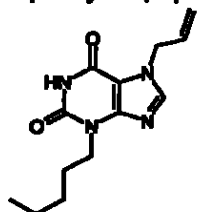
To a solution of 1-methyl-3-pentyl-7-(2-propen-1-yl)-3,7-dihydro-1H-purine-2,6-dione (1.05g, 3.8mmol) in THF (15ml) at -78°C was added lithium hexamethyldisilazide (1M solution in hexane, 4ml, 4mmol) over 10 min. The solution was stirred for 0.5h, then DMF (0.5ml, 6.5mmol) added, and the solution stirred for a further 0.5h at -78°C after which it was allowed to warm to rt over 2h. The reaction was quenched with 2N HCl solution then separated between EtOAc/brine. Chromatography over silica (gradient elution with DCM to 5:1 DCM/EtOAc) provided the title compound (0.35g, 30%). m/z 305 $[\text{MH}^+]$.

15 1-methyl-3-pentyl-7-(2-propen-1-yl)-3,7-dihydro-1H-purine-2,6-dione



To a solution of 3-pentyl-7-(2-propen-1-yl)-3,7-dihydro-1H-purine-2,6-dione (0.20g, 0.76mmol) and methyl iodide (0.5ml, 4.9mmol) in DMF (5ml) was added potassium carbonate (0.4g, 2.9mmol) and the mixture heated at 50°C for 3h, cooled and the mixture separated between EtOAc/brine. The organics were isolated, dried (MgSO_4) and concentrated to yield the required product (0.21g, 100%). m/z 277 $[\text{MH}^+]$.

3-pentyl-7-(2-propen-1-yl)-3,7-dihydro-1H-purine-2,6-dione



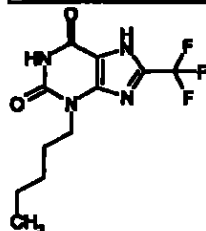
To a solution of 7-(2-propen-1-yl)-3,7-dihydro-1H-purine-2,6-dione (0.61g, 3.2mmol) and pentyl iodide (0.64g, 3.2mmol) in DMF (7ml) was added sodium carbonate (0.80g, 5.7mmol) and the mixture heated at 50°C for 18h. After cooling and the mixture was separated between EtOAc/brine. The organics were isolated, dried (MgSO_4) and concentrated to yield

the crude product which was purified by chromatography over silica (gradient elution with DCM to 5:1 DCM/EtOAc) providing the title compound (0.47g, 58%). m/z 263 $[MH^+]$.

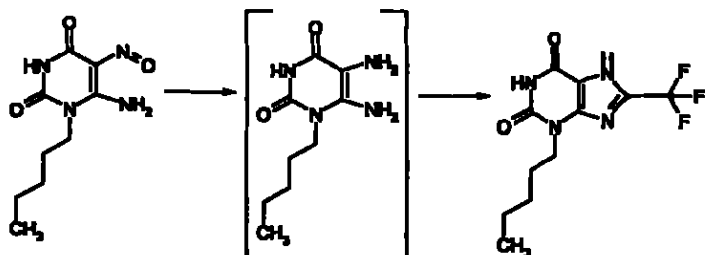
3)

Example 5

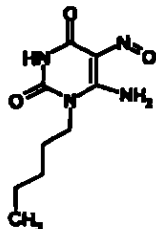
5 3-pentyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione



A mixture of 6-amino-5-nitroso-1-pentyl-2,4(1H,3H)-pyrimidinedione (500mg, 2.21mmol), 10% Pd/C (100mg) and EtOH (100ml) was vigorously stirred under an atmosphere of hydrogen for 4 hours. The mixture was filtered through Celite and the filtrate concentrated *in vacuo*, giving 5,6-diamino-1-pentyl-2,4(1H,3H)-pyrimidinedione as a pale green solid (62mg) and as a mixture with an unknown product (2:1). Trifluoroacetic anhydride was added (5ml) and heated at reflux for 1 hour then concentrated *in vacuo*. 2M NaOH(aq) (15ml) was added to the crude residue and heated at 100°C for 30 minutes. After allowing to cool to rt, the mixture was acidified to ca. pH 5 with 2M HCl(aq). The resulting precipitate was collected by filtration and then taken up into MeOH with heating and then passed down an amino propyl column (5g), eluting with 2% AcOH/MeOH, 5% AcOH/MeOH, 10% AcOH/MeOH and 15% AcOH/MeOH. The product fraction was concentrated, giving the title compound as an off-white solid (52mg). NMR; (400MHz, d_6 -DMSO) δ_H 0.85 (t, 3H, J=7Hz), 1.22-1.31 (m, 4H), 1.61-1.66 (quintet, 2H, J=7Hz), 3.89 (t, 2H, J=7Hz), 11.39 (s, 1H), 15.3 (br. s, 1H). m/z 291.2 $[MH^+]$.



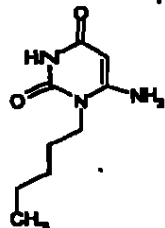
25 6-amino-5-nitroso-1-pentyl-2,4(1H,3H)-pyrimidinedione



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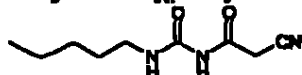
A mixture of 6-amino-1-pentyl-2,4(1*H*,3*H*)-pyrimidinedione (3.0g, 15.2mmol), AcOH (6ml) and 6M HCl(aq) was cooled to 0°C then treated dropwise with a solution of NaNO₂ (1.2g, 16.7mmol) in water (4ml). The mixture was stirred for 45 minutes and water added (ca. 200ml). The resulting purple solid was filtered, washed with water (ca. 200ml) and dried under high vacuum at 60°C for 18 hours. Gave the title compound as a purple solid (2.08g, 60%). *m/z* 227.3 [M⁺].

6-amino-1-pentyl-2,4(1*H*,3*H*)-pyrimidinedione



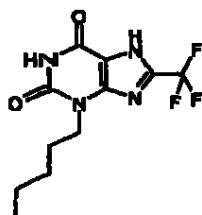
A mixture of 2-cyano-*N*-[(pentylamino)carbonyl]acetamide (17.9g, 0.091mol), EtOH (70ml) and water (40ml) was heated to 85°C and then 10% NaOH(aq) (10ml) was added dropwise. The reaction was stirred for 1 hour before allowing to cool to rt. then diluted with water and acidified to ca. pH6 using 2M HCl(aq). The mixture was cooled (ice-bath) then filtered and the resulting solid washed with water then dried under vacuum at 40°C. This gave the title compound as a white solid (11.0g, 61%). *m/z* 198.2 [M⁺].

2-cyano-*N*-[(pentylamino)carbonyl]acetamide



A mixture of pentyl urea (16.4g, 0.13mol), cyanoacetic acid (11.8g, 0.14mol) and acetic anhydride (30ml) was heated at 80°C for 2 hours. The mixture was allowed to cool to rt. and stirred with Et₂O (ca.100ml) for 1 hour. The mixture was cooled to 0°C (ice-bath) and the solid collected by filtration giving the title compound as a white solid (17.9g, 72%). *m/z* 198.1 [M⁺].

Example 5(b): 3-Pentyl-8-(trifluoromethyl)-3,7-dihydro-1*H*-purine-2,6-dione (alternative method)



A suspension of 6-amino-5-nitroso-1-pentyl-2,4(1*H*,3*H*)-pyrimidinedione (0.227g, 1mmol) in anhydrous dichloromethane (5ml) was added trifluoroacetic anhydride (0.35ml, 2.5mmol).

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The resulting pale yellow suspension was stirred for 40min at room temperature and then the solution was concentrated *in vacuo*.

The residue was dissolved in dioxan (25ml) and added to 10% palladium on carbon (0.5g) and hydrogenated for 1hr. The catalyst was filtered off, washed with dioxan and the filtrate concentrated *in vacuo*. Some starting material remained so the hydrogenation step was repeated. After a further 1.5h the catalyst was filtered off, washed with dioxan and the filtrate concentrated *in vacuo*.

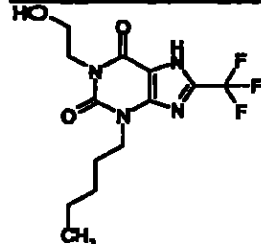
The residue was dissolved in tetrahydrofuran (10ml) and 2N NaOH (5ml) was added. The solution was stirred at 60°C for 2h. The crude reaction mixture was diluted with ethyl acetate and water and then acidified with 2N HCl. The organic phase was washed with brine, dried (MgSO₄) and concentrated *in vacuo* to give the title compound as an orange solid (140mg 48%).

LC: m/z 291 [MH]⁺, RT 3.00min

¹H NMR (DMSO-d₆) δ _H 0.85 (3H, t, J = 7Hz), 1.28 (4H, m), 1.64 (2H, m), 3.88 (2H, t, J = 7Hz), 11.22 (1H, br s).

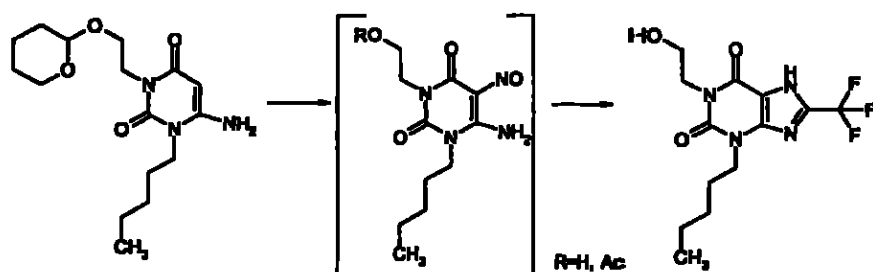
Example 6

1-(2-hydroxyethyl)-3-pentyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione

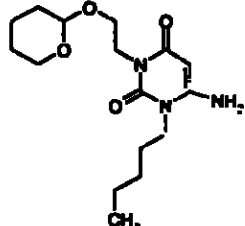


A solution of 6-amino-1-pentyl-3-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]-2,4(1H,3H)-pyrimidin-2-one (970mg) in AcOH (10ml) was treated with 6M HCl(aq) (8ml) and then a solution of NaNO₂ (247mg) in water (3ml) was added. After 15 minutes the mixture was partitioned between water and EtOAc. The organic layer was separated and the aqueous layer extracted with EtOAc (x7). The combined extracts were washed with brine, dried (MgSO₄) and concentrated, giving a purple oil (446mg). A suspension of this oil in DCM (12ml) was treated with the dropwise addition of TFAA (12ml). After 10 minutes the mixture was concentrated giving a yellow/brown oil (798mg). A portion of this material (150mg) was reacted under an atmosphere of hydrogen in THF (15ml) for 2.5 hours. The mixture was filtered through celite and washed through with THF. The filtrate was concentrated and treated once more with TFAA (5ml) and then concentrated *in vacuo*. 2M NaOH(aq) (4ml) was added to the residue and the mixture heated at 50°C for 30 minutes. After cooling, the mixture was acidified to ca. pH 5 and the product extracted with EtOAc. The organic layer was separated, washed with brine, dried (MgSO₄) and concentrated. The resulting residue was passed down an autoprep hplc to furnish the title compound (32mg, 17%). NMR; (400MHz, d₆-DMSO) δ _H 0.85 (t, 3H, J=7Hz), 1.23-1.36 (m, 4H), 1.62-1.70 (m, 2H), 3.52 (t, 2H, J=6.5Hz), 3.97 (m, 4H), 15.5 (br. S, 1H). m/z 335.3 [MH⁺].

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6-amino-1-pentyl-3-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]-2,4(1H,3H)-pyrimidinedione

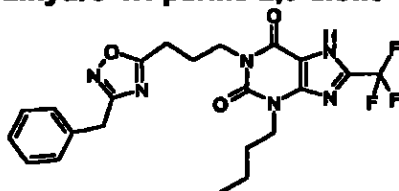


A mixture of 6-amino-1-pentyl-2,4(1H,3H)-pyrimidinedione (300mg, 1.5mmol), Cs_2CO_3 (595mg, 1.8mmol), 2-(2-bromoethoxy)tetrahydro-2H-pyran (276ul, 1.8mmol) and anhydrous DMF (5ml) was heated at 40°C for 18 hours. The mixture was partitioned between water and EtOAc. The organic layer was separated, washed with brine, dried (MgSO_4) and concentrated, giving a pale brown gum (198mg, 40%). m/z 328.3 $[\text{MH}^+]$.

Example 7(a): 3-Butyl-1-[3-[3-(phenylmethyl)-1,2,4-oxadiazol-5-yl]propyl]-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione

Example 7(b): 4-[3-Butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]butanoic acid

a) 3-Butyl-1-[3-[3-(phenylmethyl)-1,2,4-oxadiazol-5-yl]propyl]-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione



4-[3-Butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]butanoic acid (0.181g, 0.5mmol) and CDI (0.089g, 0.55mmol) were dissolved in anhydrous dimethylformamide (6ml). After stirring the solution under nitrogen for 3h at room temperature, benzylamidoxime (0.083g, 0.55mmol) was added and the solution heated at 90°C for 20h, then at 110°C for 4h. The reaction mixture was concentrated *in vacuo* to a yellow oil. This was purified on a Teledyne Isco CombiFlash® Companion® using a 40g RediSep silica cartridge (gradient elution with chloroform/ethanol 0-15%). The appropriate

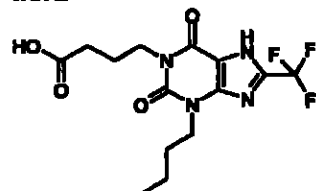
41

fractions were evaporated *in vacuo*, then freeze dried from dioxan to give the title compound as a white lyophilate (142mg, 59%)

LC/MS: m/z 477 $[MH]^+$, RT 3.57 min

1H NMR ($CDCl_3$) δ 0.98 (3H, t, $J = 7$ Hz), 1.41 (2H, m), 1.76 (2H, m), 2.23 (2H, m), 2.95 (2H, t, $J = 8$ Hz), 4.00 (2H, s), 4.14 (2H, t, $J = 8$ Hz), 4.22 (2H, t, $J = 7$ Hz), 7.27 (5H, s)

b) 4-[3-Butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]butanoic acid



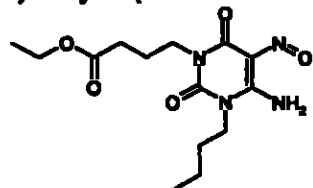
Anhydrous dichloromethane (50ml) was added to ethyl 4-(4-amino-3-butyl-5-nitroso-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)butanoate (3.26g, 10mmol), followed by trifluoroacetic anhydride (3.53ml, 25mmol). The yellow solution was stirred at room temperature under an atmosphere of nitrogen for 15min and then concentrated *in vacuo*. The residue was dissolved in dioxan (150ml) and added to 10% palladium on carbon (0.5g) and hydrogenated for 2h. The catalyst was filtered off, washed with dioxan and the filtrate concentrated *in vacuo*.

The residue was dissolved in tetrahydrofuran (100ml) and 2N NaOH (50ml) was added. The solution was stirred at 60°C for 1.5h cooled and diluted with ethyl acetate (100ml) and water (30ml). The organic phase was extracted with water (2x 30ml). The combined aqueous extracts were acidified to pH4 with 2N HCl. The resulting solid was filtered and washed with water to give the title compound as pale cream solid 2.17g (60%).

LC/MS: m/z 363 $[MH]^+$, RT 2.84min

1H NMR ($DMSO-d_6$) δ 0.90 (3H, t, $J = 7$ Hz), 1.31 (2H, m), 1.64 (2H, m), 1.80 (2H, m), 2.24 (2H, t, $J = 7$ Hz), 3.89 (2H, t, $J = 7$ Hz), 3.95 (4H, m), 12.01, (1H, br s), 15.36, (1H, br s)

c) Ethyl 4-(4-amino-3-butyl-5-nitroso-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)butanoate



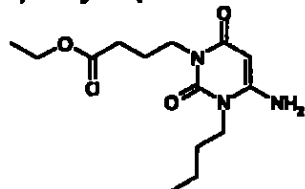
Ethyl 4-(4-amino-3-butyl-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)butanoate (8.5g, 29.8mmol) was dissolved in a mixture of glacial acetic acid (45ml) and 6M hydrochloric acid (24ml). The solution was cooled to -1°C and sodium nitrite (2.26g, 32.7mmol) in water (20ml) was added dropwise. After the addition was complete the ice bath was removed and the purple solution stirred for 1h. The solution was diluted with water (140ml) and the product, which separated out after a few minutes, was filtered, washed with water (total 350ml) and dried *in vacuo* at 50°C to give the title compound as a bright pink solid (6.61g). On standing a further crop was

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Isolated (0.53g), then the mother liquors were basified to pH6 with 2N sodium hydroxide and extracted into ethyl acetate (2x 250mL). The combined extracts were concentrated *in vacuo* to give a third crop of the title compound (1.23g). Total yield 8.37g 86%

LC/MS: m/z 327 [MH]⁺, RT 2.58 min

d) Ethyl 4-(4-amino-3-butyl-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)butanoate



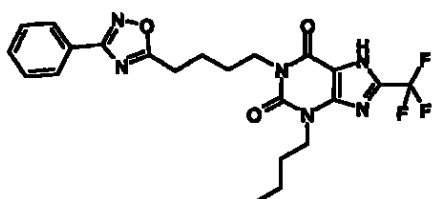
6-Amino-1-butyl-2,4(1H,3H)-pyrimidinedione (5.9g, 32.2mmol) and caesium carbonate (11.54g, 35.4mmol) were added to anhydrous dimethylformamide under a nitrogen atmosphere. Ethyl 4-bromobutyrate (5.07ml, 35.4mmol) was added and the mixture stirred at room temperature for 22h. The reaction mixture was diluted with ethyl acetate (350ml) and washed with saturated sodium chloride solution (2 x 50ml). The combined aqueous phases were back extracted with ethyl acetate. The combined organic phases were washed with saturated sodium chloride solution, dried (MgSO₄) and concentrated *in vacuo*. The crude product was purified on a Teledyne Isco CombiFlash® Companion® using a 80g RediSep silica cartridge (gradient elution using chloroform/ethanol 0-40%). The appropriate fractions were evaporated *in vacuo* to give the title compound as a yellow oil which crystallised on standing (5.36g, 56%)

LC/MS: m/z 298 [MH]⁺, RT 2.49 min

Example 8 (a): 3-Butyl-1-[4-(3-phenyl-1,2,4-oxadiazol-5-yl)butyl]-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione

Example 8 (b): 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoic acid

a) 3-Butyl-1-[4-(3-phenyl-1,2,4-oxadiazol-5-yl)butyl]-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione



A mixture of methyl 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoate (0.156g, 0.4mmol), benzamidoxime (60mg, 0.44mmol), 21% solution of NaOEt in EtOH (0.22ml, 0.6mmol) and anhydrous EtOH (1 ml) was heated in the microwave at 140°C for 10min. The mixture was diluted with EtOAc (30ml) and washed with water (20ml), 2N HCl (20ml) and brine (5ml). The organic layer was dried (Na₂SO₄) and concentrated *in*

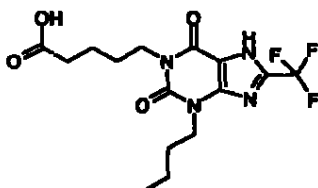
43

vacuo to a yellow oil and then freeze dried from dioxan to give the title compound as a cream lyophilate (95mg, 50%)

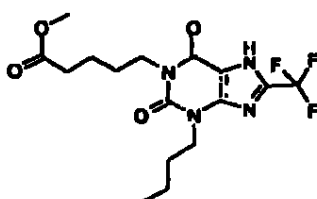
LC/MS: m/z 476 $[MH]^+$, RT 3.73 min

1H NMR ($CDCl_3$) δ 0.97 (3H, t, $J = 7$ Hz), 1.41 (2H, m), 1.77 (2H, m), 1.87 (2H, m), 1.97 (2H, m), 3.02 (2H, t, $J = 7$ Hz), 4.17 (4H, m), 7.47 (3H, m), 8.04 (2H, m)

b) 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoic acid



and methyl 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoate



15 Anhydrous dichloromethane (70ml) was added to methyl 4-(4-amino-3-butyl-5-nitroso-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)pentanoate (5.06g, 15.5mmol), followed by trifluoroacetic anhydride (22ml, 10equiv.). The yellow solution was stirred at room temperature under an atmosphere of nitrogen for 25min and then concentrated *in vacuo*.

20 The residue was dissolved in dioxan (250ml) and added to 10% palladium on carbon (0.85g) and hydrogenated for 2.25h. The catalyst was filtered off, washed with dioxan and the filtrate concentrated *in vacuo*. The residue was dissolved in tetrahydrofuran (100ml) and 2N NaOH (15.5ml) was added. The solution was stirred at room temperature for 3.5h. A further 8ml of 2N NaOH was added and the reaction mixture heated at 60°C for 1.5h. The reaction mixture was cooled and concentrated *in vacuo* to a small volume and partitioned between 25 ethyl acetate and water. The organic phase was washed with water (2x) and brine. The combined aqueous extracts were acidified to with 2N HCl, extracted into ethyl acetate (5x40ml) and then back extracted with saturated $NaHCO_3$ (3x30ml) and brine (30ml). The combined aqueous phases were acidified to pH 4.5 with 2N HCl and resulting solid was 30 filtered and washed with water to give 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoic acid as a cream solid 2.19g (38%).

The organic phase was dried over $MgSO_4$ and concentrated *in vacuo* to a yellow gum which solidified on standing to give methyl 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoate as a peach coloured solid (0.9g, 15%)

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Data for 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoic acid:

LC/MS: m/z 377 $[MH]^+$, RT 2.91 min

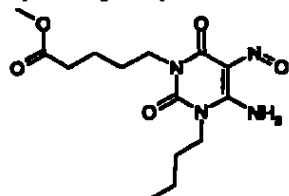
1H NMR ($DMSO-d_6$) δ_H 0.90 (3H, t, $J = 7$ Hz), 1.30 (2H, t, $J = 7$ Hz), 1.45-1.68 (6H, m), 2.24 (2H, t, $J = 7$ Hz), 3.89 (2H, t, $J = 7$ Hz), 3.97 (2H, t, $J = 7$ Hz), 12.00 (1H, br s)

Data for methyl 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoate:

LC/MS: m/z 391 $[MH]^+$, RT 3.15 min.

1H NMR ($CDCl_3$) δ_H 0.95 (3H, t, $J = 7$ Hz), 1.27 (2H, t, $J = 7$ Hz), 1.38 (2H, m), 1.75 (2H, m), 2.06 (2H, s), 2.36 (2H, t, $J = 7$ Hz), 3.62 (3H, s), 4.03 (2H, br), 4.14 (2H, m)

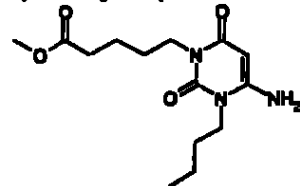
c) Methyl 4-(4-amino-3-butyl-5-nitroso-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)pentanoate



15 Prepared in similar fashion to ethyl 4-(4-amino-3-butyl-5-nitroso-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)butanoate

LC/MS: m/z 327 $[MH]^+$, RT 2.54 min

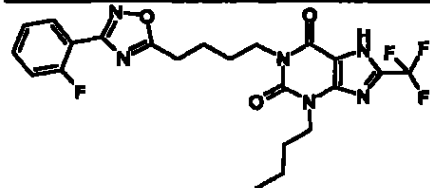
d) Methyl 5-(4-amino-3-butyl-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)pentanoate



20 Prepared in the same way as ethyl 4-(4-amino-3-butyl-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)butanoate

LC/MS: m/z 298 $[MH]^+$, RT 2.47 min

25 **Example 9: 3-Butyl-1-[4-[3-(2-fluorophenyl)-1,2,4-oxadiazol-5-yl]butyl]-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione**

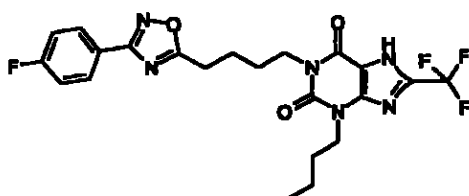


30 5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoic acid (0.301g, 0.8mmol) and CDI (0.153g, 0.94mmol) were dissolved in anhydrous dimethylformamide (10ml). After stirring the solution under nitrogen for 4h at room

temperature, 2-fluorobenzamidoxime (0.135g, 0.88mmol) was added and the solution heated at 85°C for 18h. The reaction mixture was concentrated *in vacuo* and purified on a 10g aminopropyl SPE (10g) eluting with methanol. Evaporation of the methanol eluant gave an oil that was purified further by MDAP. The relevant fractions were combined and concentrated *in vacuo* and the residue was freeze dried from dioxan to give the title compound (0.037g, 9%) as a white lyophilate. LC/MS: m/z 495 $[MH]^+$, RT 3.68 min

1H NMR ($CDCl_3$) δ_H 0.97 (3H, t, $J = 7$ Hz), 1.40 (2H, m), 1.77 (2H, m), 1.88 (2H, m), 1.97 (2H, m), 3.05 (2H, t, $J = 7$ Hz), 4.17 (2H, q, $J = 7$ Hz), 7.19-7.29 (2H, m) 7.45-7.48 (1H, m) 8.02, (1H, dt, $J = 8$ and 2Hz)

Example 10: 3-Butyl-1-(4-[3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl]butyl)-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione

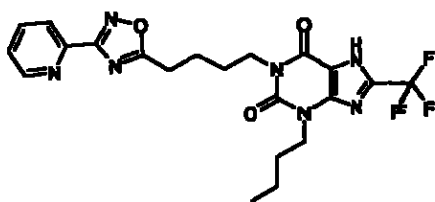


5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoic acid (0.301g, 0.8mmol) and CDI (0.153g, 0.94mmol) were dissolved in anhydrous dimethylformamide (10ml). After stirring the solution under nitrogen for 4h at room temperature 4-fluorobenzamidoxime (0.135g, 0.88mmol) was added and the solution heated at 85°C for 22h. The reaction mixture was concentrated *in vacuo*, taken up in ethyl acetate and washed with brine (2x20ml) and 0.1N HCl. The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give the required product as a pale yellow oil which crystallised on standing. This material was freeze dried from dioxan to give the title compound as a cream lyophilate (0.225g, 57%).

LC/MS: m/z 495 $[MH]^+$, RT 3.77 min

1H NMR ($CDCl_3$) δ_H 0.97 (3H, t, $J = 7$ Hz), 1.27 (2H, m), 1.77 (2H, m), 1.87 (2H, m), 1.97 (2H, m), 3.02 (2H, t, $J = 7$ Hz), 4.17 (4H, m), 7.15 (2H, m, $J = 9$ Hz), 8.04 (2H, m).

Example 11: 3-Butyl-1-(4-[3-(2-pyridinyl)-1,2,4-oxadiazol-5-yl]butyl)-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione



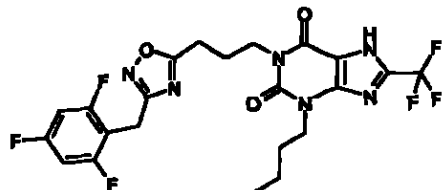
5-[3-butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]pentanoic acid (0.15g, 0.4mmol) and CDI (0.078g, 0.48mmol) were dissolved in anhydrous

dimethylformamide (5ml). After stirring the solution under nitrogen for 1.5h at room temperature 2-pyridylamidoxime (0.06g, 0.44mmol) was added and the solution heated at 90°C for 18.5h. The reaction mixture was concentrated *in vacuo* and purified by MDAIP. The relevant fractions were evaporated to give the title compound as a tan solid (0.017g, 9%).

5 LC/MS: m/z 478 $[MH]^+$, RT 3.28 min

1H NMR ($CDCl_3$) δ_H 0.95 (3H, t, $J = 7Hz$), 1.39 (2H, m, $J = 7Hz$), 1.75 (2H, m), 1.84 (2H, m), 1.98 (2H, m), 3.07 (2H, t, $J = 7Hz$), 4.14 (4H, m), 7.41 (1H, m) 7.84 (1H, dt, $J = 8$ and 2Hz), 8.10 (1H, d, $J = 8Hz$), 8.75 (1H, d, $J = 4Hz$)

10 **Example 12: 3-Butyl-8-(trifluoromethyl)-1-(3-(3-(2,4,6-trifluorophenyl)methyl)-1,2,4-oxadiazol-5-yl)propyl)-3,7-dihydro-1H-purine-2,6-dione**



15 4-[3-Butyl-2,6-dioxo-8-(trifluoromethyl)-2,3,6,7-tetrahydro-1H-purin-1-yl]butanol acid (0.181g, 0.5mmol) and CDI (0.089g, 0.55mmol) were dissolved in anhydrous dimethylformamide (8ml). After stirring the solution under nitrogen for 3h at room temperature, 2,4,6-trifluorobenzylamidoxime (0.112g, 0.55mmol) was added and the solution heated at 90°C for 20h, then at 110°C for 6h. The reaction mixture was concentrated *in vacuo* to a yellow oil. This was purified on a Teledyne Isco CombiFlash® Companion® using a 35g RediSep silica cartridge (gradient elution chloroform/ethanol 0-15%). The appropriate fractions were evaporated *in vacuo* to give after freeze drying from dioxane the title compound as a white solid (0.122g, 46%).

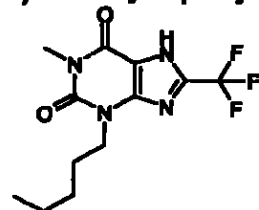
20 LC/MS: m/z 531 $[MH]^+$, RT 3.61 min

25 1H NMR ($CDCl_3$) δ_H 0.97 (3H, t, $J = 7Hz$), 1.41 (2H, m), 1.76 (2H, m), 2.23 (2H, m), 2.95 (2H, t, $J = 8Hz$), 4.00 (2H, s), 4.14 (2H, t, $J = 8Hz$), 4.22 (2H, t, $J = 7Hz$), 6.67 (2H, t, $J = 7Hz$).

Example 13: 1-Methyl-3-pentyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione

30

a) 1-Methyl-3-pentyl-8-(trifluoromethyl)-3,7-dihydro-1H-purine-2,6-dione



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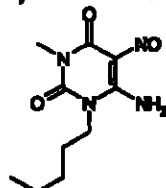
A solution of 6-amino-3-methyl-5-nitroso-1-pentyl-2,4(1H,3H)-pyrimidinedione (0.042g, 0.18mmol) in anhydrous DCM (2ml) was treated with trifluoroacetic anhydride (1ml) and left to stir at rt. for 10 mins. The solution was concentrated *in vacuo* and the residue taken up into 1,4-dioxane (15ml) and reacted with 10% Pd/C (70mg) and trifluoroacetic anhydride (0.1ml) under an atmosphere of hydrogen for 6h. The mixture was filtered through celite and the

filtrate concentrated, giving a red oil. THF (2ml) was added followed by 2M NaOH(aq) solution. The mixture was heated to 50°C for 10 mins. After cooling, the mixture was partitioned between 2M HCl(aq) and EtOAc. The organic layer was separated, washed with brine, dried (MgSO₄) and concentrated. The title product was purified by MDAP to give the title compound as a white solid (10mg, 19%).

LC/MS m/z 305 [MH⁺], RT 3.2mins.

¹H NMR; (d⁶-DMSO) δ_H 0.92 (3H, t, J = 7 Hz), 1.38 (4H, m), 1.80 (2H, m), 3.51 (3H, s), 4.17 (2H, t, J = 8Hz), 13.5 (1H, br s).

- 10 b) 6-amino-3-methyl-5-nitroso-1-pentyl-2,4(1H,3H)-pyrimidinedione



Prepared in similar fashion to ethyl 4-(4-amino-3-butyl-5-nitroso-2,6-dioxo-3,6-dihydro-1(2H)-pyrimidinyl)butanoate.

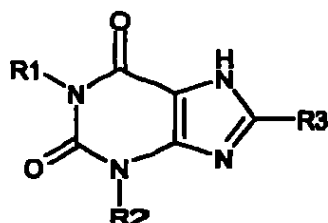
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All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

Claims

48

1. At least one chemical entity selected from compounds of formula (I)



5 (I)

and pharmaceutically acceptable derivatives thereof, wherein

10 R^1 represents a group selected from: hydrogen, C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, and $-(alk)_m-X-(alk)_n-Y$,

Wherein X represents A, A1, A2 or a direct link;

15 A represents a group selected from:
cycloalkylene, cycloalkenylene, aryl, heteroaryl, heterocyclyl, $-CH_2-OC(O)-$;

A1 represents a group selected from:
 $-CH_2-O-(CH_2)_n-aryl-O-$, $-CH_2-O-(CH_2)_n-N(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)-$,
 $-CH_2(O)_n-(CH_2)_n-C(O)NR^5-$, $-CH_2-N(R^5)C(O)N(R^5)-$, $-CH_2-C(O)N((CH_2)_nOH)-$,
 20 $-CH_2-NR^5-S(O)_2-$, $CH_2-S(O)_2NR^5-$, $-CH_2-C(O)O-$, $-O-$, $-NR^5-$, $-S-$;

A2 represents:

$-CH(OH)-$;

25 When X is A, A1 or A2, Y represents a group selected from:
heteroaryl, heterocyclyl, aryl, cycloalkyl, cycloalkenyl, $-O(CH_2)_n-aryl$, $-C(O)O-aryl$, $-CH(aryl)_2$,
 $-CH(heteroaryl)_2$, $-C_{1-4}$ haloalkyl, $-C(O)R^4$, $-NR^5R^7$, $-C(O)NR^5R^7$, $-NR^5C(O)R^7$, $-NR^5C(O)OR^7$,
 $-C(O)(CH_2)_nOR^4$, halogen, cyano, $-N(R^5)C(O)OR^7$, $-OC(O)NR^5R^6$, $-NR^5C(O)R^6$, $-OR^6$,
 $-OC(O)R^4$;

30

When X is A1 and Y is selected from:

$-O(CH_2)_n-aryl$, $-O-heteroaryl$, $-OR^6$, $-OC(O)R^6$, $-NH-aryl$, $-OC(O)NR^5R^6$,

n is an integer selected from 2, 3, 4 and 5;

35 When X is A1 and Y is $-CF_3$, or when X is A2, n is an integer selected from 1, 2, 3, 4 and 5;

When X is a direct link, Y represents a group selected from:

-C(O)(CH₂)_qOR⁵, -C(O)-aryl, -C(O)-heteroaryl, -C(O)-heterocyclyl, -heteroaryl, -heterocyclyl, -aryl, -cycloalkyl, -cycloalkenyl, -C₁₋₈ haloalkyl, -halo, -cyano, 3 or 4 ring fused system, -CH(aryl)₂, -CH(heteroaryl)₂, -OR⁵, -NR⁵R⁷, -NCOOR⁵, -(O)_pC(O)NR⁵R⁸, -NR⁵C(O)R⁸, -OR⁵, -(O)_pC(O)R⁴; 49

5

When Y incorporates a ring, that ring may be optionally substituted by one or more of:

C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, halogen, -NH₂, (CH₂)_q-NR⁵R⁷, -(CH₂)_q-(O)_p-(CH₂)_q-N(R⁵)C(O)OR⁵, -(CH₂)_q-N(R⁵)C(O)R⁵, -(CH₂)_q-(O)_p-(CH₂)_q-C(O)NR⁵R⁸, -(CH₂)_q-N(R⁵)C(O)N(R⁵)R⁸, -(CH₂)_q-C(O)N((CH₂)_mOH)R⁵, -(CH₂)_q-N(R⁵)-S(O)₂R⁸, -CH₂-S(O)₂N(R⁵)R⁸, -C₁₋₈ haloalkyl, -OCF₃, -OCH(F)₂, -OCH₂F, -COOR⁵, -OR⁵, -(R⁵)_pCN, -S(O)₂R⁸, -(CH₂)_nheteroaryl, -(CH₂)_nheterocyclyl, -(CH₂)_ncycloalkyl, -(CH₂)_ncycloalkenyl, -(CH₂)_naryl;

10

R² is selected from: hydrogen; or C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, and heteroaryl, each of which may be optionally substituted by one or more of: C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, C₁₋₈ haloalkyl, halogen, -CN, -OR⁴, -(CH₂)_nCOR⁴, -C(O)OR⁴, -OCOR⁴, -(CH₂)_nNR⁵R⁸, -(NH)_pCONR⁵R⁸, -OCONR⁵R⁷, and -NHC(O)OR⁷;

15

20 R³ is selected from: halogenated C₁₋₈ alkyl;

R⁴ is selected from: hydrogen, C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, -(CH₂)_ncycloalkyl, -(CH₂)_ncycloalkenyl, -(CH₂)_nheterocyclyl, -(CH₂)_naryl, and -(CH₂)_nheteroaryl;

25 R⁶ and R⁸ are selected from: hydrogen and C₁₋₄ alkyl;

R⁷ is selected from: hydrogen, C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, -(CH₂)_ncycloalkyl, -(CH₂)_ncycloalkenyl, -(CH₂)_nheterocyclyl, -(CH₂)_naryl, and -(CH₂)_nheteroaryl;

30 R⁹ is selected from C₁₋₄ alkyl;

R⁹ is selected from C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, -(CH₂)_ncycloalkyl, -(CH₂)_ncycloalkenyl, -(CH₂)_nheterocyclyl, -(CH₂)_naryl, and -(CH₂)_nheteroaryl, CN;

35 m represents an integer selected from: 0, 1, 2, 3, 4 and 5;

n represents an integer selected from: 0, 1, 2, 3, 4 and 5;

p represents an integer selected from: 0 and 1;

40

q represents an integer selected from: 0, 1 and 2;

t represents an integer selected from: 1 and 2;

45 w represents an integer selected from: 2, 3 and 4;

with the proviso that:

30

i) when R^1 represents hydrogen or C_{1-3} alkyl, R^2 is different to R^1 ;

ii) when R^1 represents ethyl and R^2 represents CF_3 , R^2 is other than $CH_2CH=CH_2$; and

5 iii) when R^1 represents Methyl and R^2 represents CF_3 , R^2 is other than t-butyl.

2. At least one chemical entity according to claim 1 wherein R^1 is selected from: hydrogen, C_{1-10} alkyl, and $-(alk)_m-X-(alk)_n-Y$.

10 3. At least one chemical entity according to claim 1 or 2 wherein X represents A or a direct link.

4. At least one chemical entity according to claim 1 or 2 wherein R^1 is selected from: hydrogen and C_{1-8} alkyl.

15

5. At least one chemical entity according to any preceding claim wherein R^2 is selected from: C_{4-8} alkyl.

20 6. At least one chemical entity according to any preceding claim wherein R^3 is selected from: fluorinated C_{1-8} alkyl.

7. At least one chemical entity according to any preceding claim wherein R^3 represents CF_3 .

25 8. At least one chemical entity according to any preceding claim for use in human or veterinary medicine.

9. At least one chemical entity according to any one of claims 1-7, for use in the treatment of disorders of lipid metabolism including dyslipidaemia and hyperlipoproteinaemia and/or of inflammatory diseases or conditions.

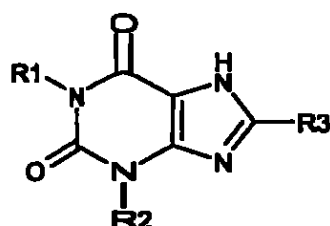
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10. At least one chemical entity according to any one of claims 1-7, for use in the treatment of diabetic dyslipidaemia, mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity, coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease or stroke.

35

51

11. At least one chemical entity selected from compounds of formula (I)



(I)

5 and pharmaceutically acceptable derivatives thereof, wherein

R¹ represents a group selected from: hydrogen, C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, and -(alk)_m-X-(alk)_n-Y,

10 Wherein X represents A, A1, A2 or a direct link;

A represents a group selected from:

cycloalkylene, cycloalkenylene, aryl, heteroaryl, heterocyclidyl, -CH₂-OC(O)-;

15 A1 represents a group selected from:

-CH₂-O-(CH₂)₄-aryl-O-, -CH₂-O-(CH₂)_w-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)-, -CH₂-(O)_p-(CH₂)_q-C(O)NR⁵-, -CH₂-N(R⁵)C(O)N(R⁵)-, -CH₂-C(O)N((CH₂)_wOH)-, -CH₂-NR⁵-S(O)_x-, CH₂-S(O)₂NR⁵-, -CH₂-C(O)O-, -O-, -NR⁵-, -S-;

20 A2 represents:

-CH(OH)-;

When X is A, A1 or A2, Y represents a group selected from:

25 heteroaryl, heterocyclidyl, aryl, cycloalkyl, cycloalkenyl, -O(CH₂)_n-aryl, -C(O)O-aryl, -CH(aryl)₂, -CH(heteroaryl)₂, -C₁₋₈ haloalkyl, -C(O)R⁴-, -NR⁵R⁷-, -C(O)NR⁵R⁷-, -NR⁵C(O)R⁷-, -NR⁵C(O)OR⁷-, -C(O)(CH₂)_qOR⁴-, halogen, cyano, -N(R⁵)C(O)OR⁷-, -OC(O)NR⁵R⁸-, -NR⁵C(O)R⁸-, -OR⁸-, -OC(O)R⁴;

When X is A1 and Y is selected from:

30 -O(CH₂)_n-aryl, -O-heteroaryl, -OR⁵-, -OC(O)R⁵-, -NH-aryl, -OC(O)NR⁵R⁸-,
n is an integer selected from 2, 3, 4 and 5;

When X is A1 and Y is -CF₃, or when X is A2, n is an integer selected from 1, 2, 3, 4 and 5;

35 When X is a direct link, Y represents a group selected from:

-C(O)(CH₂)_qOR⁴-, -C(O)-aryl, -C(O)-heteroaryl, -C(O)-heterocyclidyl, -heteroaryl, -heterocyclidyl, -aryl, -cycloalkyl, -cycloalkenyl, -C₁₋₈ haloalkyl, -halo, -cyano, 3 or 4 ring fused system,

52

$-\text{CH}(\text{aryl})_2$, $-\text{CH}(\text{heteroaryl})_2$, $-\text{OR}^6$, $-\text{NR}^6\text{R}^7$, $-\text{NCOOR}^6$, $-(\text{O})_p\text{C}(\text{O})\text{NR}^6\text{R}^6$, $-\text{NR}^6\text{C}(\text{O})\text{R}^6$, $-\text{OR}^6$, $-(\text{O})_p\text{C}(\text{O})\text{R}^6$;

When Y incorporates a ring, that ring may be optionally substituted by one or more of:

C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, halogen, $-\text{NH}_2$, $(\text{CH}_2)_q-\text{NR}^6\text{R}^7$, $-(\text{CH}_2)_q-(\text{O})_p-(\text{CH}_2)_q-\text{N}(\text{R}^6)\text{C}(\text{O})\text{OR}^6$, $-(\text{CH}_2)_q-\text{N}(\text{R}^6)\text{C}(\text{O})\text{R}^6$, $-(\text{CH}_2)_q-(\text{O})_p-(\text{CH}_2)_q-\text{C}(\text{O})\text{NR}^6\text{R}^6$, $-(\text{CH}_2)_q-\text{N}(\text{R}^6)\text{C}(\text{O})\text{N}(\text{R}^6)\text{R}^6$, $-(\text{CH}_2)_q-\text{C}(\text{O})\text{N}((\text{CH}_2)_m\text{OH})\text{R}^6$, $-(\text{CH}_2)_q-\text{N}(\text{R}^6)-\text{S}(\text{O})_2\text{R}^6$, $-\text{CH}_2-\text{S}(\text{O})_2\text{N}(\text{R}^6)\text{R}^6$, $-\text{C}_{1-6}$ haloalkyl, $-\text{OCF}_3$, $-\text{OCH}(\text{F})_2$, $-\text{OCH}_2\text{F}$, $-\text{COOR}^6$, $-\text{OR}^6$, $(\text{R}^6)_p\text{CN}$, $-\text{S}(\text{O})_2\text{R}^6$, $-(\text{CH}_2)_n\text{heteroaryl}$, $-(\text{CH}_2)_n\text{heterocycl}$, $-(\text{CH}_2)_n\text{cycloalkyl}$, $-(\text{CH}_2)_n\text{cycloalkenyl}$, $-(\text{CH}_2)_n\text{aryl}$;

R^2 is selected from: hydrogen; or C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, cycloalkyl, cycloalkenyl, heterocycl, aryl, and heteroaryl, each of which may be optionally substituted by one or more of: C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, cycloalkyl, heterocycl, aryl, heteroaryl, C_{1-6} haloalkyl, halogen, $-\text{CN}$, $-\text{OR}^4$, $-(\text{CH}_2)_n\text{COR}^4$, $-\text{C}(\text{O})\text{OR}^4$, $-\text{OCOR}^4$, $-(\text{CH}_2)_n\text{NR}^6\text{R}^6$, $-(\text{NH})_p\text{CONR}^6\text{R}^6$, $-\text{OCONR}^6\text{R}^7$, and $-\text{NHC}(\text{O})\text{OR}^7$;

R^3 is selected from: halogenated C_{1-6} alkyl;

R^4 is selected from: hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-(\text{CH}_2)_n\text{cycloalkyl}$, $-(\text{CH}_2)_n\text{cycloalkenyl}$, $-(\text{CH}_2)_n\text{heterocycl}$, $-(\text{CH}_2)_n\text{aryl}$, and $-(\text{CH}_2)_n\text{heteroaryl}$;

R^5 and R^6 are selected from: hydrogen and C_{1-4} alkyl;

R^7 is selected from: hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-(\text{CH}_2)_n\text{cycloalkyl}$, $-(\text{CH}_2)_n\text{cycloalkenyl}$, $-(\text{CH}_2)_n\text{heterocycl}$, $-(\text{CH}_2)_n\text{aryl}$, and $-(\text{CH}_2)_n\text{heteroaryl}$;

R^8 is selected from C_{1-4} alkyl;

R^9 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-(\text{CH}_2)_n\text{cycloalkyl}$, $-(\text{CH}_2)_n\text{cycloalkenyl}$, $-(\text{CH}_2)_n\text{heterocycl}$, $-(\text{CH}_2)_n\text{aryl}$, and $-(\text{CH}_2)_n\text{heteroaryl}$, CN;

m represents an integer selected from: 0, 1, 2, 3, 4 and 5;

n represents an integer selected from: 0, 1, 2, 3, 4 and 5;

p represents an integer selected from: 0 and 1;

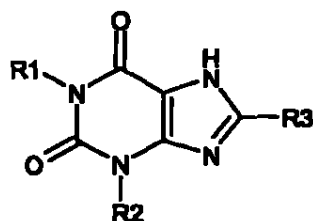
q represents an integer selected from: 0, 1 and 2;

t represents an integer selected from: 1 and 2;

w represents an integer selected from: 2, 3 and 4

for use in the manufacture of a medicament for treating diabetic dyslipidaemia, mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity, coronary artery disease, thrombosis, angina, chronic renal failure or stroke.

12. A method for the treatment of a human or animal subject having a condition where under-activation of the HM74A receptor contributes to the condition or where activation of the receptor will be beneficial, which method comprises administering to said human or animal subject an effective amount of at least one chemical entity selected from: compounds of formula (II)



(II)

and pharmaceutically acceptable derivatives thereof, wherein

10

R^1 represents a group selected from: hydrogen, C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, and $-(alk)_m-X-(alk)_n-Y$,

- 15 Wherein X represents A, A1, A2 or a direct link;

A represents a group selected from:

cycloalkylene, cycloalkenylene, aryl, heteroaryl, heterocyclyl, $-CH_2-OC(O)-$;

- 20 A1 represents a group selected from:

$-CH_2-O-(CH_2)_n-aryl-O-$, $-CH_2-O-(CH_2)_n-N(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)-$, $-CH_2-(O)_p-(CH_2)_qC(O)NR^6-$, $-CH_2-N(R^5)C(O)N(R^6)-$, $-CH_2-C(O)N((CH_2)_wOH)-$, $-CH_2-NR^5-S(O)_2-$, $CH_2-S(O)_2NR^6-$, $-CH_2-C(O)O-$, $-O-$, $-NR^5-$, $-S-$;

- 25 A2 represents:

$-CH(OH)-$;

When X is A, A1 or A2, Y represents a group selected from:

- heteroaryl, heterocyclyl, aryl, cycloalkyl, cycloalkenyl, $-O(CH_2)_n-aryl$, $-C(O)O-aryl$, $-CH(aryl)_2$, $-CH(heteroaryl)_2$, $-C_{1-8}$ haloalkyl, $-C(O)R^4$, $-NR^5R^7$, $-C(O)NR^5R^7$, $-NR^5C(O)R^7$, $-NR^5C(O)OR^7$, $-C(O)(CH_2)_kOR^4$, halogen, cyano, $-N(R^5)C(O)OR^7$, $-OC(O)NR^5R^6$, $-NR^5C(O)R^6$, $-OR^6$, $-OC(O)R^4$;

- When X is A1 and Y is selected from:

- 35 $-O(CH_2)_n-aryl$, $-O-heteroaryl$, $-OR^6$, $-OC(O)R^6$, $-NH-aryl$, $-OC(O)NR^5R^6$,
n is an integer selected from 2, 3, 4 and 5;

5A

When X is A1 and Y is $-\text{CF}_3$, or when X is A2, n is an integer selected from 1, 2, 3, 4 and 5;

When X is a direct link, Y represents a group selected from:

$-\text{C}(\text{O})(\text{CH}_2)_q\text{OR}^5$, $-\text{C}(\text{O})\text{-aryl}$, $-\text{C}(\text{O})\text{-heteroaryl}$, $-\text{C}(\text{O})\text{-heterocyclyl}$, $-\text{heteroaryl}$, $-\text{heterocyclyl}$,
 5 $-\text{aryl}$, $-\text{cycloalkyl}$, $-\text{cycloalkenyl}$, $-\text{C}_{1-6}\text{haloalkyl}$, $-\text{halo}$, $-\text{cyano}$, 3 or 4 ring fused system,
 $-\text{CH}(\text{aryl})_2$, $-\text{CH}(\text{heteroaryl})_2$, $-\text{OR}^5$, $-\text{NR}^5\text{R}^7$, $-\text{NCOOR}^5$, $-(\text{O})_p\text{C}(\text{O})\text{NR}^5\text{R}^6$, $-\text{NR}^5\text{C}(\text{O})\text{R}^6$, $-\text{OR}^5$,
 $-(\text{O})_p\text{C}(\text{O})\text{R}^4$;

When Y incorporates a ring, that ring may be optionally substituted by one or more of:

10 $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{2-6}\text{alkenyl}$, $\text{C}_{2-6}\text{alkynyl}$, halogen, $-\text{NH}_2$, $(\text{CH}_2)_q\text{-NR}^5\text{R}^7$,
 $-(\text{CH}_2)_q(\text{O})_p-(\text{CH}_2)_q\text{-N}(\text{R}^5)\text{C}(\text{O})\text{OR}^5$, $-(\text{CH}_2)_q\text{-N}(\text{R}^5)\text{C}(\text{O})\text{R}^5$, $-(\text{CH}_2)_q(\text{O})_p-(\text{CH}_2)_q\text{-C}(\text{O})\text{NR}^5\text{R}^6$,
 $-(\text{CH}_2)_q\text{-N}(\text{R}^5)\text{C}(\text{O})\text{N}(\text{R}^5)\text{R}^6$, $-(\text{CH}_2)_q\text{-C}(\text{O})\text{N}((\text{CH}_2)_m\text{OH})\text{R}^5$, $-(\text{CH}_2)_q\text{-N}(\text{R}^5)\text{-S}(\text{O})_2\text{R}^5$, $-\text{CH}_2\text{-}$
 $\text{S}(\text{O})_2\text{N}(\text{R}^5)\text{R}^6$, $-\text{C}_{1-6}\text{haloalkyl}$, $-\text{OCF}_3$, $-\text{OCH}(\text{F})_2$, $-\text{OCH}_2\text{F}$, $-\text{COOR}^5$, $-\text{OR}^5$, $-(\text{R}^5)_p\text{CN}$, $-\text{S}(\text{O})_2\text{R}^5$,
 15 $-(\text{CH}_2)_n\text{heteroaryl}$, $-(\text{CH}_2)_n\text{heterocyclyl}$, $-(\text{CH}_2)_n\text{cycloalkyl}$, $-(\text{CH}_2)_n\text{cycloalkenyl}$, $-(\text{CH}_2)_n\text{aryl}$;

15 R^2 is selected from: hydrogen; or $\text{C}_{1-10}\text{alkyl}$, $\text{C}_{2-10}\text{alkenyl}$, $\text{C}_{2-10}\text{alkynyl}$, cycloalkyl ,
 cycloalkenyl , heterocyclyl , aryl , and heteroaryl , each of which may be optionally substituted
 by one or more of: $\text{C}_{1-10}\text{alkyl}$, $\text{C}_{2-10}\text{alkenyl}$, $\text{C}_{2-10}\text{alkynyl}$, cycloalkyl , heterocyclyl , aryl ,
 heteroaryl , $\text{C}_{1-6}\text{haloalkyl}$, halogen, $-\text{CN}$, $-\text{OR}^4$, $-(\text{CH}_2)_n\text{COR}^4$, $-\text{C}(\text{O})\text{OR}^4$, $-\text{OCOR}^4$,
 20 $(\text{CH}_2)_n\text{NR}^5\text{R}^6$, $-(\text{NH})_p\text{CONR}^5\text{R}^6$, $-\text{OCONR}^5\text{R}^7$, and $-\text{NHC}(\text{O})\text{OR}^7$;

R^3 is selected from: halogenated $\text{C}_{1-6}\text{alkyl}$;

25 R^4 is selected from: hydrogen, $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{2-6}\text{alkenyl}$, $\text{C}_{2-6}\text{alkynyl}$, $-(\text{CH}_2)_n\text{cycloalkyl}$,
 $-(\text{CH}_2)_n\text{cycloalkenyl}$, $-(\text{CH}_2)_n\text{heterocyclyl}$, $-(\text{CH}_2)_n\text{aryl}$, and $-(\text{CH}_2)_n\text{heteroaryl}$;

R^5 and R^6 are selected from: hydrogen and $\text{C}_{1-4}\text{alkyl}$;

30 R^7 is selected from: hydrogen, $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{2-6}\text{alkenyl}$, $\text{C}_{2-6}\text{alkynyl}$, $-(\text{CH}_2)_n\text{cycloalkyl}$, $-(\text{CH}_2)_n$
 cycloalkenyl , $-(\text{CH}_2)_n\text{heterocyclyl}$, $-(\text{CH}_2)_n\text{aryl}$, and $-(\text{CH}_2)_n\text{heteroaryl}$;

R^8 is selected from $\text{C}_{1-4}\text{alkyl}$;

35 R^9 is selected from $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{2-6}\text{alkenyl}$, $\text{C}_{2-6}\text{alkynyl}$, $-(\text{CH}_2)_n\text{cycloalkyl}$,
 $-(\text{CH}_2)_n\text{cycloalkenyl}$, $-(\text{CH}_2)_n\text{heterocyclyl}$, $-(\text{CH}_2)_n\text{aryl}$, and $-(\text{CH}_2)_n\text{heteroaryl}$, CN ;

m represents an integer selected from: 0, 1, 2, 3, 4 and 5;

n represents an integer selected from: 0, 1, 2, 3, 4 and 5;

40 p represents an integer selected from: 0 and 1;

q represents an integer selected from: 0, 1 and 2;

45 t represents an integer selected from: 1 and 2;

w represents an integer selected from: 2, 3 and 4.

55

13. A method according to claim 12 wherein the human or animal subject has a disorder of lipid metabolism including dyslipidaemia or hyperlipoproteinaemia or an inflammatory disease or condition.

5 14. A pharmaceutical formulation comprising at least one chemical entity according to any one of claims 1-7 and one or more pharmaceutically acceptable diluents, excipients or carriers.

10 15. A combination for administration together or separately, sequentially or simultaneously in separate or combined pharmaceutical formulations, said combination comprising at least one chemical entity according to any one of claims 1-7 together with another therapeutically active agent.

16. A pharmaceutical formulation comprising:

- 15 (i) At least one chemical entity according to any one of claims 1-7;
(ii) one or more active ingredients selected from statins, fibrates, bile-acid binding resins and nicotinic acid; and
(iii) one or more pharmaceutically acceptable diluents, excipients or carriers.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/011376A. CLASSIFICATION OF SUBJECT MATTER
C07D473/04 C07D473/06 A61K31/522 A61P9/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P

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

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

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KRAMER G L ET AL: "Selective inhibition of cyclic nucleotide phosphodiesterases by analogues of 1-methyl-3-isobutylxanthine" BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 16, no. 15, 1977, pages 3316-3321, XP002169138 ISSN: 0006-2960 Compound 12 ----- -/-	1,2,4, 6-14

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claims or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to art oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"A" document member of the same patent family

Date of the actual completion of the international search

26 January 2006

Date of mailing of the international search report

22/02/2006

Name and mailing address of the ISA/
European Patent Office, P.O. Box 18 Patentstr. 2
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Authorized officer

De Jong, B

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2005/011376

(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WELLS, JACK N. ET AL: "Inhibition of separated forms of cyclic nucleotide phosphodiesterase from pig coronary arteries by 1,3-disubstituted and 1,3,8-trisubstituted xanthines" JOURNAL OF MEDICINAL CHEMISTRY, 24(8), 954-8 CODEN: JMCNAR; ISSN: 0022-2623, 1981, XP002169139 compound 4	1,2,4,5, 8-14
X	JACOBSON K A ET AL: "Effect of trifluoromethyl and other substituents on activity of xanthines at adenosine receptors" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol. 36, 1993, pages 2639-2644, XP002327691 ISSN: 0022-2623 compounds 22,31	1, 14
X	CORSANO, STEFANO ET AL: "Structure-activity relationships in a series of 8-substituted xanthines as bronchodilator and A1-adenosine receptor antagonists" ARCHIV DER PHARMAZIE (WEINHEIM, GERMANY), 328(9), 654-8 CODEN: ARPMAS; ISSN: 0365-6233, 1995, XP008058976 compound 4	1,2,4,5
X	REGNIER G L ET AL: "New xanthine derivatives with potent and long lasting anti-bronchoconstrictive activity" EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 22, 1987, pages 243-250, XP002291236 ISSN: 0223-5234 compounds 39-41,45,47,49-52	1,2,4,5
X	EP 0 103 497 A (ADIR) 21 March 1984 (1984-03-21) compounds of formula (II) page 14; claim 14	1,2,4,5
X	BREDERECK, HELLMUT ET AL: "Syntheses in the purine series. XIII. The preparation of several xanthine-8-aldehydes" CHEMISCHE BERICHTE, 95, 403-13 CODEN: CHBEAM; ISSN: 0009-2940, 1962, XP002364488 compound III	1,2,4,5
X	US 2 879 271 A (KALLISCHNIGG ROLF) 24 March 1959 (1959-03-24) column 6, line 4	1,2,4
	-/-	

Form PCT/ISA/210 (continuation of annex sheet) (April 2003)

INTERNATIONAL SEARCH REPORT

oral application No
PCI/EP2005/011376

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/084298 A (GLAXO GROUP LIMITED; FOORD, STEVEN, MICHAEL; PIKE, NICHOLAS, BRIAN; WI) 24 October 2002 (2002-10-24) cited in the application page 1 - page 6	1-16

28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/011376

Box II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

Although claims 12,13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/011376

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0103497	A	21-03-1984	AU 560809 B2	16-04-1987
			AU 1736183 A	02-02-1984
			CA 1207766 A1	15-07-1986
			DD 216934 A5	02-01-1985
			DE 3362022 D1	13-03-1986
			DK 342883 A	29-01-1984
			ES 8500275 A1	01-01-1985
			FR 2531085 A1	03-02-1984
			GR 78891 A1	02-10-1984
			HU 188353 B	28-04-1986
			IE 55773 B1	16-01-1991
			IL 69362 A	31-07-1986
			JP 1692068 C	27-08-1992
			JP 3059903 B	12-09-1991
			JP 59042383 A	08-03-1984
			KR 8701268 B1	30-06-1987
			MA 19860 A1	01-04-1984
			NO 832742 A	30-01-1984
			NZ 205035 A	11-04-1986
			OA 7508 A	31-03-1985
			PH 19275 A	21-02-1986
			PT 77110 A	01-08-1983
			SU 1240361 A3	23-06-1986
			SU 1245261 A3	15-07-1986
			US 4548820 A	22-10-1985
			ZA 8305496 A	28-03-1984
US 2879271	A	24-03-1959	NONE	
WO 02084298	A	24-10-2002	EP 1377834 A2	07-01-2004
			US 2004254224 A1	16-12-2004

DERIVADOS DE XANTINA CON ACTIVIDAD DE RECEPTOR DE HM74A

CAMPO DE LA INVENCION

5 La presente invención se refiere a compuestos terapéuticamente
activos que son derivados de xantina, procedimientos para la fabricación de
dichos derivados, formulaciones farmacéuticas que contienen los compuestos
activos y el uso de los compuestos en terapia, particularmente en el
tratamiento de enfermedades en donde la sub-activación del receptor de
10 HM74A contribuye a la enfermedad o en donde la activación del receptor será
benéfica.

ANTECEDENTES DE LA INVENCION

15 La dislipidemia es un término general usado para describir a
individuos con perfiles de lipoproteína aberrantes. Clínicamente, las
principales clases de compuestos usados para el tratamiento de pacientes con
dislipidemia, y por lo tanto en riesgo de enfermedad cardiovascular son las
estatinas, fibratos, resinas de unión de ácido biliar y ácido nicotínico. El ácido
20 nicotínico (niacina, una vitamina B) se ha usado clínicamente durante
aproximadamente 40 años en pacientes con varias formas de dislipidemia. El
modo de acción primario de ácido nicotínico es por inhibición de triglicérido
lipasa sensible a hormona (HSL), que da por resultado una reducción de

ácidos grasos no esterificados en el plasma (NEFA) que a su vez alteran el metabolismo de la grasa hepática para reducir la producción de LDL y VLDL (lipoproteína de densidad baja y muy baja). Se piensa que los niveles reducidos de VLDL reducen la actividad de la proteína de transferencia de éster de colesterol inferior (CETP) para dar por resultado los niveles incrementados de HDL (lipoproteína de densidad alta) que pueden ser la causa de los beneficios cardiovasculares observados. Por lo tanto, el ácido nicotínico produce una alteración muy deseable en perfiles de lipoproteína; reduce los niveles de VLDL y LDL mientras incrementa la HDL. También se ha demostrado que el ácido nicotínico tiene beneficios modificadores de enfermedad, reduciendo la progresión e incrementando la regresión de lesiones ateroscleróticas y reduciendo el número de eventos cardiovasculares en varios ensayos.

La inhibición observada de HSL por tratamiento con ácido nicotínico es mediada por una disminución de monofosfato de adenosina cíclica (AMPc) celular causada por la inhibición mediada por proteína G de adenilil ciclasa. Recientemente, los receptores acoplados a proteína G, HM74 y HM74A, han sido identificados por receptores para ácido nicotínico (solicitud de patente del PCT WO02/84298; Wise et. al. J Biol Chem., 2003, 278 (11), 9869-9874). La secuencia de ADN de HM74A humano se puede encontrar en el banco de datos Genbank; número de acceso AY148884. Dos documentos adicionales soportan este descubrimiento (Tunaru et. al. Nature Medicine, 2003, 9(3), 352-255 y Soga et. al. Biochem Biophys Res Commun., 2003, 303

(1) 364-369), sin embargo, la nomenclatura difiere ligeramente. En el documento de Tunaru, lo que denominan HM74 humano es de hecho HM74A y en el documento de Soga, HM74b es idéntico a HM74A. Las células transfectadas para expresar HM74A y/o HM74 ganan la capacidad para
5 inducir respuestas mediadas por proteína-G Gi después de la exposición a ácido nicotínico. En ratones que carecen del homólogo de HM74A (m-PUMA-G), el ácido nicotínico no reduce los niveles de NEFA en el plasma.

Los inventores de la presente ahora presentan un grupo de derivados de xantina que son agonistas selectivos del receptor de ácido
10 nicotínico, HIV174A, y por lo tanto son de beneficio en el tratamiento, profilaxis y supresión de enfermedades en donde la sub-activación de este receptor contribuye a la enfermedad o en donde la activación del receptor será benéfica.

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BREVE DESCRIPCION DE LA INVENCION

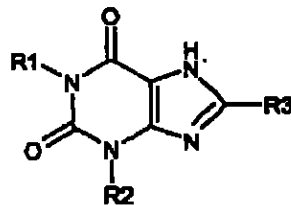
La presente invención provee derivados de xantina terapéuticamente activos y el uso de estos derivados en terapia, particularmente en el tratamiento de enfermedades en donde bajo activación
20 del receptor de HM74A contribuye a la enfermedad o en donde la activación del receptor será benéfica, en particular enfermedades del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteinemia tal como dislipidemia diabética y dislipidemia mixta, insuficiencia cardiaca, hipercolesteremia,

enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis e hipertrigliceridemia. Como tales, los compuestos también encuentran utilidad como terapéuticos para enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica y accidente cerebrovascular, así como las indicaciones cardiovasculares asociadas con diabetes mellitas de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidaemia, anorexia nerviosa y obesidad. Los compuestos también pueden usarse en el tratamiento de enfermedades o condiciones inflamatorias, como se expone más adelante.

Los intermediarios, formulaciones, métodos y procedimientos descritos aquí de aspectos adicionales de la invención.

DESCRIPCION DETALLADA DE LA INVENCION

De conformidad con un aspecto de esta invención, los inventores de la presente proveen por lo menos una entidad química seleccionada de compuestos de la fórmula (I)



(I)

y derivados farmacéuticamente aceptables de los mismos, en donde

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R^1 representa un grupo seleccionado de: hidrógeno, alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , y $-(alq)_m-X-(alq)_n-Y$,

en donde X representa A, A1, A2 o un enlace directo;

A representa un grupo seleccionado de: cicloalquileno, cicloalquenileno, arilo, heteroarilo, heterociclilo, $-CH_2-OC(O)-$;

A1 representa un grupo seleccionado de: $-CH_2-O-(CH_2)_q\text{arilo}-O-$, $-CH_2-O-(CH_2)_wN(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)-$, $-CH_2-(O)_p-(CH_2)_qC(O)NR^5-$, $-CH_2-N(R^5)C(O)N(R^5)-$, $-CH_2-C(O)N((CH_2)_wOH)-$, $-CH_2-NR^5-S(O)_2-$, $CH_2-S(O)_2NR^5-$, $-CH_2-C(O)O-$, $-O-$, $-NR^5-$, $-S-$;

A2 representa:

$-CH(OH)-$;

cuando X es A, A1 o A2, Y representa un grupo seleccionado de:

heteroarilo, heterociclilo, arilo, cicloalquilo, cicloalquenilo, $-O(CH_2)_n\text{-arilo}$, $-C(O)O\text{-arilo}$, $-CH(\text{arilo})_2$, $-CH(\text{heteroarilo})_2$, halogenoalquilo de C_{1-8} , $-C(O)R^4$, $-NR^5R^7$, $-C(O)NR^5R^7$, $-NR^5C(O)R^7$, $-NR^5C(O)OR^7$, $-C(O)(CH_2)_qOR^4$, halógeno, ciano, $-N(R^5)C(O)OR^7$, $-OC(O)NR^5R^8$, $-NR^5C(O)R^8$, $-OR^5$, $-OC(O)R^4$;

cuando X es A1 y Y se selecciona de:

$-O(CH_2)_n\text{-arilo}$, $-O\text{-heteroarilo}$, $-OR^5$, $-OC(O)R^5$, $-NH\text{-arilo}$, $-OC(O)NR^5R^8$, n es un entero seleccionado de 2, 3, 4 y 5;

cuando X es A1 y Y es $-CF_3$, o cuando X es A2, n es un entero seleccionado de 1, 2, 3, 4 y 5;

cuando X es un enlace directo, Y representa un grupo

seleccionado de:

-C(O)(CH₂)_qOR⁵, -C(O)-arilo, -C(O)-heteroarilo, -C(O)-heterociclilo, -heteroarilo, -heterociclilo, -arilo, -cicloalquilo, -cicloalquenilo, halogenoalquilo de C₁₋₈, -halógeno, -ciano, 3 ó 4 sistemas fusionados de anillo, -CH(arilo)₂, -CH(heteroarilo)₂, -OR⁵, -NR⁵R⁷, -NCOOR⁸, -
 5 (O)_pC(O)NR⁵R⁸, -MR⁵C(O)R⁸, -OR⁵, - (O)_pC(O)R⁴;

cuando Y incorpora un anillo, ese anillo puede ser opcionalmente sustituido por uno o más de:

alquilo de C₁₋₈, alquenilo de C₂₋₈, alquinilo de C₂₋₈, halógeno, -
 10 NH₂, (CH₂)_q-NR⁵R⁷, -(CH₂)_q-(O)_p-(CH₂)_q-N(R⁵)C(O)OR⁸, -(CH₂)_q-N(R⁵)C(O)R⁸,
 -(CH₂)_q-(O)_p-(CH₂)_q-C(O)NR⁵R⁸, -(CH₂)_q-N(R⁵)C(O)N(R⁵)R⁸, -(CH₂)_q-
 C(O)N((CH₂)_mOH)R⁵, -(CH₂)_q-N(R⁵)-S(O)₂R⁸, -CH₂-S(O)₂N(R⁵)R⁸, -
 halogenoalquilo de C₁₋₈, -OCF₃, -OCH(F)₂, -OCH₂F, -COOR⁵, -OR⁵, -(R⁸)_pCN, -
 S(O)₂R⁸, -(CH₂)_nheteroarilo, -(CH₂)_nheterociclilo -(CH₂)_ncicloalquilo, -
 15 (CH₂)_ncicloalquenilo, -(CH₂)_narilo;

R² se selecciona de: hidrógeno; o alquilo de C₁₋₁₀, alquenilo de C₂₋₁₀, alquinilo de C₂₋₁₀, cicloalquilo, cicloalquenilo, heterociclilo, arilo, y heteroarilo, cada uno de los cuales puede ser opcionalmente sustituido por uno o más de: alquilo de C₁₋₁₀, alquenilo de C₂₋₁₀, alquinilo de C₂₋₁₀,
 20 cicloalquilo, heterociclilo, arilo, heteroarilo, C₁₋₈ halogenoalquilo, halógeno, -CN, -OR⁴, -(CH₂)_nCOR⁴, -C(O)OR⁴, -OCOR⁴, -(CH₂)_nNR⁵R⁸, -(NH)_pCON R⁵R⁸, -OCONR⁵R⁷, y -NHC(O)OR⁷;

R³ se selecciona de: alquilo de C₁₋₈ halogenado;

R⁴ se selecciona de: hidrógeno, alquilo de C₁₋₆, alquenilo de C₂₋₆, alquinilo de C₂₋₆, -(CH₂)_n cicloalquilo, -(CH₂)_n cicloalquenilo, -(CH₂)_n heterociclilo, -(CH₂)_n arilo, y -(CH₂)_n heteroarilo;

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R⁵ y R⁶ se seleccionan de: hidrógeno y alquilo de C₁₋₄;

5 R⁷ se selecciona de: hidrógeno, alquilo de C₁₋₆, alquenilo de C₂₋₆, alquinilo de C₂₋₆, -(CH₂)_t cicloalquilo, -(CH₂)_n cicloalquenilo, -(CH₂)_t heterociclilo, -(CH₂)_t arilo, y -(CH₂)_t heteroarilo;

R⁸ se selecciona de alquilo de C₁₋₄;

10 R⁹ se selecciona de alquilo de C₁₋₆, alquenilo de C₂₋₆, alquinilo de C₂₋₆, -(CH₂)_n cicloalquilo, -(CH₂)_n cicloalquenilo, -(CH₂)_n heterociclilo, -(CH₂)_n arilo, y -(CH₂)_n heteroarilo, CN;

m representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5;

n representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5;

p representa un entero seleccionado de: 0 y 1;

15 q representa un entero seleccionado de: 0, 1 y 2;

t representa un entero seleccionado de: 1 y 2;

w representa un entero seleccionado de: 2, 3 y 4;

con la condición de que:

20 i) cuando R¹ representa hidrógeno o alquilo de C₁₋₃, R² es diferente a R¹;

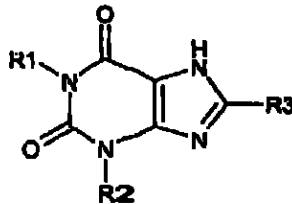
ii) cuando R¹ representa etilo y R³ representa CF₃, R² es otro distinto de CH₂-CH=CH₂; y

iii) cuando R¹ representa metilo y R³ representa CF₃, R² es otro

distinto de i-butilo.

En una modalidad de esta invención, los inventores de la presente proveen por lo menos una entidad química seleccionada de compuestos de la fórmula (I)

5



(I)

y derivados farmacéuticamente aceptables de los mismos, en

10 donde

R¹ representa hidrógeno o un grupo seleccionado de: alquilo de C₁₋₁₀, alquenilo de C₂₋₁₀, alquinilo de C₂₋₁₀, -(CH₂)_ncicloalquilo, -(CH₂)_nheterociclilo, -(CH₂)_narilo y -(CH₂)_nheteroarilo, cada uno de los cuales puede ser opcionalmente sustituido por uno o más de: -(CH₂)_ncicloalquilo, -(CH₂)_nheterociclilo, -(CH₂)_narilo, -(CH₂)_nheteroarilo, halogenoalquilo de C₁₋₈, halógeno, ciano, -OR⁴, -(CH₂)_nCOR⁴, -CO₂R⁴, -OCOR⁴, -(CH₂)_nNR⁵R⁷, -(NH)_mCONR⁵R⁷, -OCONR⁵R⁷, y -NHCO₂R⁷;

15

R² se selecciona de: alquilo de C₁₋₁₀, alquenilo de C₂₋₁₀, alquinilo de C₂₋₁₀, -(CH₂)_ncicloalquilo, -(CH₂)_nheterociclilo, -(CH₂)_narilo y -(CH₂)_nheteroarilo, cada uno de los cuales puede ser opcionalmente sustituido por uno o más de: cicloalquilo, heterociclilo, arilo, heteroarilo, halogenoalquilo de C₁₋₈, halógeno, ciano, -OR⁴, -(CH₂)_nCOR⁴, -CO₂R⁴, -OCOR⁴, -(CH₂)_nNR⁵R⁶, -(NH)_mCONR⁵R⁶, -OCONR⁵R⁷, y -NHCO₂R⁷;

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R^3 se selecciona de: alquilo de C_{1-6} halogenado;

R^4 se selecciona de: hidrógeno, alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_m$ cicloalquilo, $-(CH_2)_n$ heterociclilo, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo;

5 R^5 y R^6 se seleccionan independientemente de: hidrógeno y alquilo de C_{1-4} ;

R^7 se selecciona de: hidrógeno, alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_p$ cicloalquilo, $-(CH_2)_p$ heterociclilo, $-(CH_2)_p$ arilo, y $-(CH_2)_p$ heteroarilo;

10 n representa un entero seleccionado de: 0, 1, 2, 3 y 4;

m representa un entero seleccionado de: 0 y 1;

p representa un entero seleccionado de: 1 y 2.

con la condición de que:

i) cuando R^1 representa H o alquilo de C_{1-3} , R^2 es diferente a R^1 ;

15 ii) cuando R^1 representa etilo y R^3 representa CF_3 , R^2 es otro distinto de $CH_2-CH=CH_2$; y

iii) cuando R^1 representa metilo y R^3 representa CF_3 , R^2 es otro distinto de i-butilo.

20 A lo largo de la presente especificación y las reivindicaciones anexas, las palabras "comprende" e "incluye" y variaciones tales como "comprende", "que comprende", "incluye" y "que incluye" han de interpretarse en forma inclusiva. Es decir, se pretende que estas palabras transmitan la posible inclusión de otros elementos o entidades no mencionadas

específicamente, en donde lo permite el contexto.

Como se usa aquí, los términos "halógeno" o "halo" se refieren a flúor, cloro, bromo y yodo.

Como se usa aquí, el término "alquilo" (cuando se usa como un grupo o como parte de un grupo) se refiere a una cadena de hidrocarburo de cadena recta o ramificada a menos que se especifique de otra manera, que contiene el número especificado de átomos de carbono. Por ejemplo, alquilo de C_3 - C_{10} significa una cadena de hidrocarburo recta o ramificada que contiene por lo menos 3 y cuando mucho 10 átomos de carbono. Ejemplos de alquilo como se usa aquí incluyen, pero no se limitan a metilo (Me), etilo (Et), n-propilo e i-propilo.

Como se usa aquí, el término "alquenilo" se refiere a una cadena de hidrocarburo recta o ramificada que contiene el número especificado de átomos de carbono que contiene uno o más dobles enlaces. Ejemplos adecuados incluyen pero no se limitan a etenilo, 2-propenilo, 3-butenilo, 2-butenilo, 2-pentenilo, 3-pentenilo, 3-metil-2-butenilo, 3-hexenilo y similares.

Como se usa aquí, el término "alquinilo" se refiere a una cadena de hidrocarburo recta o ramificada que contiene el número especificado de átomos de carbono que contiene uno o más triples enlaces. Ejemplos adecuados incluyen pero no se limitan a acetilenilo, propinilo, 1-butinilo, 1-pentinilo, 3-metil-1-butinilo y similares.

El término 'halogenoalquilo de C_{1-6} ', como se usa aquí, se refiere a un grupo alquilo de C_{1-6} como se define aquí en donde por lo menos un

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átomo de hidrógeno es reemplazado por halógeno. Ejemplos de dichos grupos incluyen fluoroetilo, trifluorometilo o trifluoroetilo y similares.

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Como se usa aquí, el término "cicloalquilo" se refiere a un anillo de hidrocarburo, que contiene entre 3 y 6 átomos de carbono, que no comprende heteroátomos o dobles enlaces conjugados. Ejemplos de cicloalquilo como se usa aquí incluyen, pero no se limitan a ciclopropilo y ciclohexilo.

El término 'cicloalquileno', como se usa aquí, se refiere a un anillo de hidrocarburo monocíclico saturado de grupos enlazadores de 3 a 8 carbonos. Ejemplos de dichos grupos incluyen ciclopropileno, ciclobutileno, ciclopentileno, ciclohexileno, cicloheptileno o ciclooctileno y similares.

El término 'cicloalquenilo', como se usa aquí, se refiere a un anillo de hidrocarburo monocíclico no aromático insaturado de 3 a 8 átomos de carbono que contiene uno o más dobles enlaces carbono-carbono. Ejemplos de dichos grupos incluyen ciclopropenilo, ciclobutenilo, ciclopentenilo, ciclohexenilo, cicloheptenilo o ciclooctenilo y similares.

El término 'cicloalquenileno', como se usa aquí, se refiere a un anillo de hidrocarburo monocíclico no aromático insaturado de grupos enlazadores de 3 a 8 carbonos que contiene dobles enlaces carbono-carbono. Ejemplos de dichos grupos incluyen ciclopropenileno, ciclobutenileno, ciclopentenileno, ciclohexenileno, cicloheptenileno o ciclooctenileno y similares.

Como se usa aquí, el término "arilo" se refiere a un grupo

aromático monocíclico de 5 ó 6 miembros, o un grupo aromático bicíclico de 8-10 miembros fusionado con por lo menos un anillo que tiene un sistema de electrón pi conjugado, que contiene hasta dos sistemas de anillo conjugados o fusionados. "Ariilo" incluye grupos ariilo y biarilo carbocíclico. Ejemplos
5 adecuados incluyen pero no se limitan a fenilo, naftilo y similares.

Como se usa aquí, el término "heteroarilo" se refiere a un grupo aromático monocíclico de 5 ó 6 miembros o un grupo aromático bicíclico de 8-10 miembros fusionado que contiene 1 a 3 heteroátomos independientemente seleccionado de oxígeno, nitrógeno y azufre con por lo menos un anillo que
10 tiene un sistema de electrón pi conjugado, que contiene hasta dos sistemas de anillo conjugado o fusionado. Ejemplos adecuados de dichos anillos aromáticos monocíclicos incluyen pero no se limitan a tienilo, furilo, pirrolilo, triazolilo, imidazolilo, oxazolilo, tiazolilo, oxadiazolilo, isotiazolilo, isoxazolilo, tiadiazolilo, pirazolilo, pirimidilo, piridazinilo, pirazinilo y piridilo. Ejemplos
15 adecuados de dichos anillos aromáticos fusionados incluyen pero no se limitan a anillos aromáticos benzofusionados tales como quinolinilo, isoquinolinilo, quinazolinilo, quinoxalinilo, cinnolinilo, naftridinilo, indolilo, indazolilo, pirrolopiridinilo, benzofuranilo, benzotienilo, benzimidazolilo, benzoxazolilo, benzisoxazolilo, benzotiazolilo, bencisotiazolilo,
20 benzoxadiazolilo, benzotiadiazolilo y similares.

Como se usa aquí, el término "heterociclilo" se refiere a un grupo hidrocarburo cíclico saturado de 5 ó 6 miembros, que contiene 1 a 3 heteroátomos independientemente seleccionado de oxígeno, nitrógeno y

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azufre. Ejemplos adecuados incluyen pero no se limitan a pirrolidinilo, morfolinilo, imidazolidinilo y piperazinilo.

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El término 'sistema fusionado de anillo de 3 ó 4' como se usa aquí se refiere a un anillo tricíclico o tetracíclico de 13 a 18 miembros fusionado que contiene 1 a 4 heteroátomos de N y en donde por lo menos un anillo es aromático. Puede haber uno o más sustituyentes oxo opcionales en los átomos de carbono del anillo. Ejemplos de dichos anillos aromáticos fusionados incluyen carbazolilo, acenaftilo, naftotriazolilo y similares.

Como se usa aquí, en donde un grupo es referido como que es "sustituido" por otro grupo o que tiene "uno o más sustituyentes" a menos que una posición particular para dicha sustitución es especificada cabe entender que una sustitución puede estar presente en cualquier posición en el grupo.

Como se usa aquí, el término "derivado farmacéuticamente aceptable" se refiere a cualquier derivado farmacéuticamente aceptable de un compuesto de la presente invención, por ejemplo sales, solvatos o ésteres que, al administrarse a un mamífero, tal como un humano, son capaces de proveer (directamente o indirectamente) a compuesto de la fórmula (I) o un metabolito activo o residuo del mismo. Dichos derivados son claros para los expertos en la técnica, sin experimentación extraordinaria, y con referencia a las enseñanzas de Burger's Medicinal Chemistry And Drug Discovery, 5ª. Edición, Vol 1: Principles And Practice, que se incorpora aquí por referencia.

Ahora, los expertos en la técnica apreciarán que los compuestos de la invención pueden ser modificados para proveer derivados

farmacéuticamente aceptables de los mismos en cualesquiera de los grupos funcionales en los compuestos, y que los compuestos de la invención pueden ser así modificados en más de una posición.

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En una modalidad, "derivado farmacéuticamente aceptable" se refiere a sales o solvatos.

Como se usa aquí, el término "farmacéuticamente aceptable" usado en relación con un ingrediente (ingrediente o excipiente activo) que se pueden incluir en una formulación farmacéutica para administrarse a un paciente, se refiere a ese ingrediente que es aceptable en el sentido de ser compatible con cualesquiera otros ingredientes presentes en la formulación farmacéutica y sin ser deletéreo al receptor del mismo. Como se usa aquí, el término "solvato" se refiere a un complejo de estequiometría variable formada por un soluto (en esta invención, un compuesto de la fórmula (I), una sal del mismo o un derivado farmacéuticamente aceptable del mismo) y un solvente. Dichos solventes para los propósitos de la presente invención pueden no interferir con la actividad biológica del soluto. El solvente usado puede ser un solvente farmacéuticamente aceptable. Ejemplos de solventes farmacéuticamente aceptables adecuados incluyen agua, etanol y ácido acético. Un ejemplo de un solvente que se puede usar es agua, en cuyo caso el solvato puede referirse como un hidrato del soluto en cuestión.

Se apreciará que, para uso farmacéutico, la "sal o solvato" referida anteriormente será una sal o solvato farmacéuticamente aceptable. Sin embargo, otras sales o solvatos se pueden encontrar, por ejemplo, en la

preparación de un compuesto de la fórmula (I) o en la preparación de una sal solvato farmacéuticamente aceptable del mismo.

Las sales farmacéuticamente aceptables incluyen aquellas descritas por Berge, Bighley y Monkhouse, J. Pharm. Sci., 1977, 66, 1-19. Las sales farmacéuticamente aceptables adecuadas incluyen sales de metal alcalino formadas de la adición de bases de metal alcalino tales como hidróxidos de metal alcalino. Ejemplos de sales de metal alcalino adecuadas son sal de sodio o sal de potasio. Otras sales farmacéuticamente aceptables adecuadas incluyen sales de metal alcalinotérreo tales como sal de calcio o sal de magnesio, sales de amonio; o sales con bases orgánicas tales como etanolamina, trietanolamina, etileno diamina, trietilamina, colina y meglumina; o sales con aminoácidos tales como arginina, lisina y histidina.

Los compuestos se usan en el tratamiento de enfermedades en donde la sub-activación del receptor de HM74A contribuye a la enfermedad o en donde activación del receptor será benéfica, en particular enfermedades de metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteínaemia tales como dislipidemia diabética y dislipidemia mixta, insuficiencia cardíaca, hipercolesteraemia, enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis, e hipertrigliceridemia. Como tal, los compuestos también pueden tener utilidad como terapéuticos para enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica y accidente cerebrovascular, así como las indicaciones cardiovasculares asociadas con diabetes mellitus de tipo II, diabetes de tipo I, resistencia a la

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insulina, hiperlipidemia, anorexia nerviosa, obesidad. Como tales, los compuestos de la presente invención pueden encontrar uso como agonistas o agonistas parciales de HM74A.

Los compuestos de la invención son de beneficio terapéutico

5 potencial en el tratamiento y alivio de los síntomas de muchas enfermedades de metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteinaemia tales como dislipidemia diabética y dislipidemia mixta, insuficiencia cardíaca, hipercolesteraemia, enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis, e hipertrigliceridaemia, diabetes mellitus de tipo II, diabetes de

10 tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad. Como tales, los compuestos también pueden ser favorecidos como terapéuticos para enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica y accidente cerebrovascular. Además, también se cree que los receptores de HM74 y

15 HM74A están implicados en inflamación. La inflamación representa un grupo de respuestas vasculares, celulares y neurológicas a inflamación por trauma. La inflamación se puede caracterizar por el movimiento de células inflamatorias tales como monocitos, neutrófilos y granulocitos en los tejidos. Esto está usualmente asociado con una función de barrera endotelial reducida

20 y edema en los tejidos. La inflamación con respecto a enfermedad típicamente se refiere como inflamación crónica y puede durar hasta de por vida. Dicha inflamación crónica puede manifestarse a través de síntomas de la enfermedad. La finalidad de terapia anti-inflamatoria es por lo tanto reducir

esta inflamación crónica y permitir que progrese el proceso fisiológico de cicatrización y reparación de tejido.

Ejemplos de enfermedades o condiciones inflamatorias para las cuales los compuestos de la presente invención pueden demostrar utilidad incluyen las de las articulaciones, particularmente artritis (v.gr., artritis reumatoide, osteoartritis, insuficiencia de articulación prostética), o el tracto gastrointestinal (v.gr., colitis ulcerativa, enfermedad de Crohn, y otras enfermedades intestinales y gastrointestinales inflamatorias, gastritis e inflamación de la mucosa que resultan de infección, la enteropatía provocada por fármacos anti-inflamatorios no esteroideos), del pulmón (v.gr., síndrome de sufrimiento respiratorio en adultos, asma, fibrosis cística, o enfermedad pulmonar obstructiva crónica), del corazón (v.gr., miocarditis), de tejido nervioso (v.gr., esclerosis múltiple), del páncreas, (v.gr., inflamación asociada con diabetes mellitus y complicaciones de la misma, del riñón (v.gr., glomerulonefritis), de la piel (v.gr., dermatitis, psoriasis, eczema, urticaria, lesión por quemadura), de los ojos (v.gr., glaucoma) así como de órganos trasplantados (v.gr., rechazo) y enfermedades de órganos múltiples (v.gr., lupus eritematoso sistémico, sepsia) y secuelas inflamatorias de infecciones virales o bacterianas y condiciones inflamatorias asociadas con aterosclerosis y después de ataques hipóxico o isquémico (con o sin perfusión), por ejemplo en el cerebro o en enfermedad cardíaca isquémica.

Se apreciará que las referencias aquí a tratamiento se extienden a profilaxis, prevención de recurrencia y supresión de síntomas así como el

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tratamiento de condiciones establecidas.

El ácido nicotínico tiene un perfil de efecto colateral significativo, posiblemente debido a que es dosificado a un nivel alto (cantidades en gramos diarias). El efecto colateral más común es un rubor cutáneo intenso.

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5 En ciertas modalidades de la presente invención los compuestos pueden exhibir efectos colaterales reducidos comparados con ácido nicotínico. HM74A se ha identificado como un receptor de alta afinidad para ácido nicotínico mientras que HM74 es un receptor de afinidad más baja. Los compuestos de la presente invención pueden usarse como agonistas de HM74A selectivos o
10 agonistas parciales; en cuyo caso mostrarán afinidad mayor para HM74A que para HM74.

El potencial para compuestos de la fórmula (I) para activar HM74A se puede demostrar, por ejemplo, usando las siguientes pruebas enteras *in vitro*:

15

Pruebas *in vitro*

Para transfecciones transitorias, células HEK293T (células HEK293 expresan establemente el antígeno T grande de SV40) se mantuvieron en DMEM que contenía 10% de suero de ternera fetal y 2 mM de
20 glutamina. Las células se sembraron en cajas de cultivo de 90 mm y se hicieron crecer hasta 60-80% de confluencia (18-24 hr) antes de la transfección. HM74A humanas (número de acceso al GenBank™ AY148884) se incubaron en un vector de expresión de mamífero (pcDNA3; Invitrogen) y se

transfectaron usando reactivo de lipofectamina. Para transfección, 9 µg de ADN se mezcló con 30 µl de lipofectamina en 0.6 ml de Opti-MEM (Life Technologies Inc.) y se incubó a temperatura ambiente durante 30 min antes de la adición de 1.6 ml de Opti-MEM. Las células se expusieron a la mezcla de lipofectamina/ADN durante 5 hr y 6 ml de 20% (v/v) de suero de ternera fetal en DMEM se añadió después. Las células se cosecharon 48 hr después de la transfección. El tratamiento de toxina de Pertussis se llevó a cabo por complementación en el medio a 50 ngmr¹ durante 16 hr. Todos los estudios transitorios implicaron co-transfección de receptor junto con la proteína G₁₀ G, G₀₁ α.

Para la generación de líneas de células estables, el método anterior se usó para transfectar células CHO-K1 sembradas en cajas con seis pozos y se hicieron crecer hasta 30% de confluencia. Estas células se mantuvieron en un medio DMEM F-12 HAM que contenía 10% de suero de ternera fetal y 2 mM de glutamina. 48 hr después de la transfección, el medio se complementó con 400 µg/ml de Geneticina (G418, Gibco) para selección de células resistentes a antibiótico. Las líneas de células CHO-K1 clonales que expresaban establemente HM74A fueron confirmadas por mediciones de unión a [³⁵S]-GTPyS, después de la adición de ácido nicotínico.

Preparación de membrana – Membrana plasmática que contenía fracciones de partícula de P2 se prepararon a partir de pastas de células congeladas a -80°C después de la cosecha. Todos los procedimientos se llevaron a cabo a 4°C. Las pastillas de células se volvieron a suspender en 1

ml de Tris-HCl 10 mM y EDTA 0.1 mM, pH 7.5 (regulador de pH A) y por
homogeneización durante 20 seg con un Ultra Turrax seguido por el paso (5
veces) a través de una aguja calibre 25. Los lisados de células se
centrifugaron a 1,000 g durante 10 min en una microcentrifuga para formar
5 una pastilla de los núcleos y células no rotas y fracciones de partículas de P2
se recuperaron por microcentrifugación a 16,000 g durante 30 min. Las
fracciones de partículas de P2 se volvieron a suspender en regulador de pH A
y se almacenaron a -80°C hasta que se requirió.

Unión a [³⁵S]-GTPyS – Se realizaron pruebas a temperatura
10 ambiente en un formato de 384 pozos basadas en métodos descritos
anteriormente (Wieland, T. y Jakobs, K.H. (1994) Methods Enzymol. 237, 3-
13). En breve, la adición de compuestos estándares o de prueba se
prepararon y se añadieron a una placa de 384 pozos en un volumen de 10 µl.
Las membranas (HM74A o HM74) se diluyeron en regulador de pH de prueba
15 (20 mM de HEPES, 100 mM de NaCl, 10 mM de MgCl₂, pH 7.4)
complementado con saponina (60 µg/ml), esferas Leadseeker WGA
(Amersham; 250 µg/pozo) y 10 µM GDP, por lo que un volumen de 20 µl
añadido a cada pozo contenía 5 µg de membranas. [³⁵S]-GTPyS (1170
Ci/mmol, Amersham) se diluyó (1:1500) en regulador de pH de prueba y 20 µl
20 se añadieron a cada pozo. Después de la adición del radioligando, las placas
se sellaron, se agitaron por pulsos y se incubaron durante 4 horas a
temperatura ambiente. Al final del período de incubación, las placas se
leyeron en una máquina Leadseeker (VIEWLUX PLUS; Perkin-Elmer) para

determinar los niveles de unión específica.

Estas pruebas se refinaron reduciendo el volumen de prueba final a 10 µl. Para esta prueba de 10 µl se usó un protocolo revisado. Esto implicó el uso de únicamente 100 nl de compuesto estándar o de prueba por
5 pozo de una placa de 384 pozos y 1.5 µg de membrana y 100 µg de esferas de Leadseeker WGA. Para el protocolo de volumen bajo, la membrana, esferas y [³⁵S]-GTPyS se mezclaron entre sí y después 10 µl de esta mezcla se suministraron a cada pozo. La incubación y lectura de la placa fueron idénticas para las pruebas de 10 µl y 50 µl.

10 Todos los compuestos ilustrados se probaron en una o ambas de las pruebas de unión a [³⁵S]-GTPyS anteriormente descritas (es decir, las pruebas de 10 µl y 50 µl).

Los datos se analizaron por ajuste de la curva como se lleva a cabo usando una ecuación logística de cuatro parámetros usando el paquete
15 de software XC50 (máx 2 puntos suprimidos de cualquier otra curva). La unión específica se expresa como pEC₅₀ y como % de eficacia comparada con la respuesta máxima de unión a ácido nicotínico.

Prueba *in vivo*

20 Los agonistas de HM74A se pueden probar en ratas Spague-Dawley macho (200-250 g) que se han dejado en ayunas por lo menos durante 12 horas antes del estudio. Los compuestos son dosificados por vía intravenosa ya sea 1 o 3 mg/kg (5 ml/kg) o por alimentación forzada oral a

dosis que varían de 1-30 mg/kg (10 ml/kg). Se pueden tomar muestras de sangre (0.3 ml de sangre de la vena de la cola) antes de la dosis y tres veces después de la dosis (las veces varían de 15 minutos a 6 horas después de la dosis). Cada muestra de sangre es transferida a un tubo de heparina (Becton Dickinson Microtainer, PST LH) y centrifugada (10,000 g durante 5 minutos) para producir una muestra de plasma. Las muestras de plasma se prueban para niveles de ácidos grasos no esterificados (NEFA) usando un equipo comercialmente disponible (Randox). La inhibición de los niveles de plasma NEFA, en relación con los niveles de pre-dosis, se usan como un sustituto para actividad agonista de HM74A.

Para determinar si los compuestos de HM74A presentan la respuesta al rubor asociada con ácido nicotínico, pueden ser dosificadas a cobayos concientes. Cobayos Dunkin Hartley machos (300-600 g; n=10-20 por grupo) se dejan en ayunas por lo menos durante 12 horas, pero no en exceso de 24 horas antes de la experimentación. Se tomaron muestras de sangre de pre-estudio (0.5 ml) de cada animal por punción cardíaca bajo anestesia de recuperación (Isoflurano al 3.5% con O₂ adicional (1 l/min)). Las mediciones de temperatura de la oreja se toman colocando la oreja izquierda de cada animal sobre una sonda de temperatura infrarroja. Las mediciones se toman a intervalos de un minuto de 5 minutos antes de la dosis a 30 minutos después de la dosis. Las mediciones de temperatura se toman después a intervalos de 15 minutos hasta 2 horas después de la dosis. Los animales reciben compuestos de prueba por alimentación forzada oral (5 ml/kg). Se

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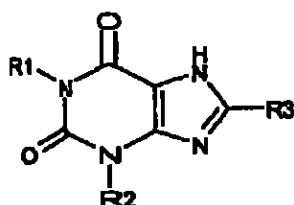
toman muestras de sangre (0.5 ml) por punción cardiaca bajo anestesia terminal. Se toman muestras de sangre de animales individuales para proveer datos a 0.5, 1, 2, 3 y 4 horas después de la dosis. Todas las muestras de sangre se colocan en un rodillo para sangre durante 5 minutos y después se
5 almacenan sobre hielo hasta el final del estudio. Después de centrifugar (12000 g durante 5 min) el plasma es transferido a tubos frescos y almacenado a -20°C hasta que se prueba para concentraciones de NEFA.

Los compuestos de conformidad con la fórmula (I) se han sintetizado (véase ejemplos sintéticos más adelante) y se prueban en las
10 pruebas de unión a [³⁵S]-GTPγS anteriormente descritas. Todos los compuestos ilustrados tienen una pEC₅₀ de 4.3 o mayor y una eficacia de 30% o mayor (en relación con ácido nicotínico).

Los compuestos de la fórmula (I) pueden usarse en medicina humana o veterinaria, en particular como activadores de HM74A, en el manejo
15 de dislipidemia e hiperlipoproteinemia.

De conformidad con otro aspecto de la invención, se provee el uso de por lo menos una entidad química seleccionada de compuestos de la fórmula (II)

20



(II)

y derivados farmacéuticamente aceptables de los mismos, en

donde

R^1 representa un grupo seleccionado de: hidrógeno, alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , y $-(alq)_m-X-(alq)_n-Y$,

en donde X representa A, A1, A2 o un enlace directo;

5 A representa un grupo seleccionado de: cicloalquileno, cicloalquenileno, arilo, heteroarilo, heterociclilo, $-CH_2-OC(O)-$;

A1 representa un grupo seleccionado de: $-CH_2-O-(CH_2)_q$ arilo-O-,
 $-CH_2-O-(CH_2)_wN(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)O-$, $-CH_2-N(R^5)C(O)-$, $-CH_2-(O)_p-$
 $(CH_2)_qC(O)NR^5-$, $-CH_2-N(R^5)C(O)N(R^5)-$, $-CH_2-C(O)N((CH_2)_wOH)-$, $-CH_2-NR^5-$
 10 $S(O)_2-$, $CH_2-S(O)_2NR^5-$, $-CH_2-C(O)O-$, $-O-$, $-NR^5-$, $-S-$;

A2 representa:

$-CH(OH)-$;

cuando X es A, A1 o A2, Y representa un grupo seleccionado de:
 heteroarilo, heterociclilo, arilo, cicloalquilo, cicloalquenilo, $-O(CH_2)_n$ arilo, -
 15 $C(O)O$ arilo, $-CH(arilo)_2$, $-CH(heteroarilo)_2$, halogenoalquilo de C_{1-8} , $-C(O)R^4$, -
 NR^5R^7 , $-C(O)NR^5R^7$, $-NR^5C(O)R^7$, $-NR^5C(O)OR^7$, $-C(O)(CH_2)_qOR^4$, halógeno,
 ciano, $-N(R^5)C(O)OR^7$, $-OC(O)NR^5R^6$, $-NR^5C(O)R^6$, $-OR^5$, $-OC(O)R^4$;

cuando X es A1 y Y se selecciona de:

$-O(CH_2)_n$ arilo, $-O$ -heteroarilo, $-OR^5$, $-OC(O)R^5$, $-NH$ -arilo, -
 20 $OC(O)NR^5R^6$, n es un entero seleccionado de 2, 3, 4 y 5;

cuando X es A1 y Y es $-CF_3$, o cuando X es A2, n es un entero
 seleccionado de 1, 2, 3, 4 y 5;

cuando X es un enlace directo, Y representa un grupo

seleccionado de: $-C(O)(CH_2)_qOR^5$, $-C(O)$ -arilo, $-C(O)$ -heteroarilo, $-C(O)$ -heterociclilo, $-heteroarilo$, $-heterociclilo$, $-arilo$, $-cicloalquilo$, $-cicloalquenilo$, halogenoalquilo de C_{1-8} , $-halógeno$, $-ciano$, 3 ó 4 sistemas fusionados de anillo, $-CH(arilo)_2$, $-CH(heteroarilo)_2$, $-OR^5$, $-NR^5R^7$, $-NCOOR^8$,
 5 $(O)_pC(O)NR^5R^8$, $-MR^5C(O)R^8$, $-OR^5$, $-(O)_pC(O)R^4$;

cuando Y incorpora un anillo, ese anillo puede ser opcionalmente sustituido por uno o más de: alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , $halógeno$, $-NH_2$, $(CH_2)_q-NR^5R^7$, $-(CH_2)_q-(O)_p-(CH_2)_q-N(R^5)C(O)OR^8$, $-(CH_2)_q-N(R^5)C(O)R^8$, $-(CH_2)_q-(O)_p-(CH_2)_q-C(O)NR^5R^8$, $-(CH_2)_q-N(R^5)C(O)N(R^5)R^8$,
 10 $(CH_2)_q-C(O)N((CH_2)_mOH)R^5$, $-(CH_2)_q-N(R^5)-S(O)_2R^8$, $-CH_2-S(O)_2N(R^5)R^8$, $-halogenoalquilo$ de C_{1-8} , $-OCF_3$, $-OCH(F)_2$, $-OCH_2F$, $-COOR^5$, $-OR^5$, $-(R^8)_pCN$, $-S(O)_2R^9$, $-(CH_2)_n$ heteroarilo, $-(CH_2)_n$ heterociclilo $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_n$ arilo;

R^2 se selecciona de: hidrógeno; o alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, cicloalquenilo, heterociclilo, arilo, y heteroarilo, cada uno de los cuales puede ser opcionalmente sustituido por uno o más de: alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, heterociclilo, arilo, heteroarilo, C_{1-8} halogenoalquilo, $halógeno$, $-CN$, $-OR^4$, $-(CH_2)_nCOR^4$, $-C(O)OR^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^8$, $-(NH)_pCONR^5R^8$,
 15 $-OCONR^5R^7$, y $-NHC(O)OR^7$;

R^3 se selecciona de: alquilo de C_{1-8} halogenado;

R^4 se selecciona de: hidrógeno, alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_n$

heterociclilo, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo;

R^5 y R^6 se seleccionan de: hidrógeno y alquilo de C_{1-4} ;

R^7 se selecciona de: hidrógeno, alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_t$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_t$ heterociclilo, $-(CH_2)_t$ arilo, y $-(CH_2)_t$ heteroarilo;

R^8 se selecciona de alquilo de C_{1-4} ;

R^9 se selecciona de alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_n$ heterociclilo, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo, CN;

10 m representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5;

n representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5;

p representa un entero seleccionado de: 0 y 1;

q representa un entero seleccionado de: 0, 1 y 2;

t representa un entero seleccionado de: 1 y 2;

15 w representa un entero seleccionado de: 2, 3 y 4;

en la fabricación de un medicamento para el tratamiento de trastornos del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteinemia. En particular, se provee el uso de un compuesto de fórmula (II) en la fabricación de un medicamento para el tratamiento de dislipidemia diabética o dislipidemia mixta, insuficiencia cardiaca, hipercolesteremia, diabetes mellitus de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad, enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, accidente

cerebrovascular y enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis e hipertrigliceridemia.

En una modalidad de la invención, se provee un compuesto de la fórmula (I) o (II) o un derivado farmacéuticamente aceptable del mismo para
5 usarse en el tratamiento de trastornos del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteinemia. En otra modalidad, se provee el uso de un compuesto de la fórmula (I) o (II) en la fabricación de un medicamento para el tratamiento de dislipidemia diabética o dislipidemia mixta, insuficiencia
cardiaca, hipercolesteraemia, diabetes mellitus de tipo II, diabetes de tipo I,
10 resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad, enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, accidente cerebrovascular y enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis e hipertrigliceridemia.

Por lo tanto, se provee como un aspecto adicional de la presente
15 invención a compuesto de la fórmula (I) o (II) o un derivado farmacéuticamente aceptable del mismo, para usarse en medicina humana o veterinaria, particularmente en el tratamiento de trastornos del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteínaemia tal como dislipidemia diabética y dislipidemia mixta, insuficiencia cardiaca, hipercolesteraemia,
20 enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis, e hipertrigliceridemia, diabetes mellitus de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad. Como tales, los compuestos también se proveen para usarse en el tratamiento de enfermedad

de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica y accidente cerebrovascular.

Se provee como un aspecto adicional de la presente invención un compuesto de la fórmula (I) o (II) o un derivado farmacéuticamente aceptable del mismo, para usarse en la fabricación de un medicamento para el tratamiento de trastornos del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteínaemia tal como dislipidemia diabética y dislipidemia mixta, insuficiencia cardíaca, hipercolesterolemia, enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis e hipertrigliceridemia, diabetes mellitus de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad. Como tales, los compuestos también se proveen para usarse en el tratamiento de enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica y accidente cerebrovascular.

En una modalidad adicional, la presente invención provee el uso de un compuesto de la fórmula (I) o (II) o un derivado farmacéuticamente aceptable del mismo, en la fabricación de un medicamento para el tratamiento de enfermedades o condiciones inflamatorias de las articulaciones, particularmente artritis (v.gr., artritis reumatoide, osteoartritis, insuficiencia de articulaciones protética), o del tracto gastrointestinal (v.gr., colitis ulcerativa, enfermedad de Crohn, y otras enfermedades intestinales y gastrointestinal inflamatorias, gastritis e inflamación de la mucosa que resultan de infección, la enteropatía provocada por fármacos anti-inflamatorios no esteroideos), del

pulmón (v.gr., síndrome de sufrimiento respiratorio en adultos, asma, fibrosis
cística, o enfermedad pulmonar obstructiva crónica), del corazón (v.gr.,
miocarditis), de tejido nervioso (v.gr., esclerosis múltiple), del páncreas, (v.gr.,
inflamación asociada con diabetes mellitus y complicaciones de la misma, del
5 riñón (v.gr., glomerulonefritis), de la piel (v.gr., dermatitis, psoriasis, eczema,
urticaria, lesión por quemadura), de los ojos (v.gr., glaucoma) así como de
órganos trasplantados (v.gr., rechazo) y enfermedades de órganos múltiples
(v.gr., lupus eritematoso sistémico, sepsia) y secuelas inflamatorias de
infecciones virales o bacterianas y condiciones inflamatorias asociadas con
10 aterosclerosis y después de ataques hipóxico o isquémico (con o sin
reperfusión), por ejemplo en el cerebro o en enfermedad cardíaca isquémica.

En una modalidad, los compuestos de la fórmula (I) o (II) o
derivados farmacéuticamente aceptables del mismo son útiles en el
tratamiento y prevención de inflamación, diabetes y enfermedades o
15 condiciones cardiovasculares incluyendo aterosclerosis, arteriosclerosis,
hipertrigliceridemia, y dislipidemia mixta.

En un aspecto adicional o alternativo se provee un método para
el tratamiento de un sujeto humano o animal con una condición en donde la
subactivación del receptor de HM74A contribuye a la condición o en donde la
20 activación del receptor será benéfica, el método comprende administrar a
dicho sujeto humano o animal una cantidad efectiva de un compuesto de la
fórmula (I) o (II) o a derivado farmacéuticamente aceptable del mismo.

En una modalidad, la presente invención provee un método para

el tratamiento de trastornos del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteinaemia tal como dislipidemia diabética y dislipidemia mixta, insuficiencia cardíaca, hipercolesteraemia, enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis, e hipertrigliceridemia, diabetes mellitus de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad, dicho método comprende administrar a dicho sujeto humano o animal una cantidad efectiva de un compuesto de la fórmula (I) o (II) o un derivado farmacéuticamente aceptable del mismo. Como tales, estos compuestos también pueden favorecer en métodos para el tratamiento de enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica y accidente cerebrovascular, dichos métodos comprenden administrar a dicho sujeto humano o animal una cantidad efectiva de a compuesto de la fórmula (I) o (II) o un derivado farmacéuticamente aceptable del mismo.

En una modalidad de la fórmula (I) o (II), R^1 se selecciona de hidrógeno C_{1-10} alquilo, y $-(alq)_m-X-(alq)_n-r$.

En una modalidad de la fórmula (I) o (II), X representa A o A1. En una modalidad adicional de la fórmula (I) o (II), A se selecciona de heteroarilo, heterociclilo, y A1 se selecciona de $CH_2-O-(CH_2)_wN(R^5)C(O)O-$, por ejemplo, $CH_2O(CH_2)_2NHC(O)O-$, $CH_2-N(R^5)C(O)O-$, por ejemplo, $CH_2-NHC(O)O$, $CH_2-N(R^5)C(O)-$, por ejemplo, $CH_2-NHC(O)-$, $CH_2-(O)_p-(CH_2)_qC(O)NR^5-$, por ejemplo, $CH_2C(O)NCH_3-$, $CH_2-N(R^5)C(O)N(R^5)-$, por ejemplo, $CH_2-NHC(O)NCH_3-$, $CH_2-C(O)N((CH_2)_wOH)-$, por ejemplo, $CH_2-C(O)N((CH_2)_2OH)-$,

CH₂-NR⁵-S(O)₂- por ejemplo CH₂-NH-S(O)₂-, CH₂-S(O)₂NR⁵-, por ejemplo, CH₂-S(O)₂NCH₃-, y CH₂-C(O)O-. En otra modalidad de la fórmula (I) o (II), X representa A y A representa un heteroarilo. En otra modalidad de la fórmula (I) o (II), A representa un heteroarilo que comprende un heteroátomo de nitrógeno, por ejemplo, triazolilo, furazanilo, oxadiazolilo, tetrazolilo, imidazolilo o pirazolilo.

En una modalidad adicional de la fórmula (I) o (II), X representa A, por ejemplo, heteroarilo o heterociclilo, o un enlace directo.

En una modalidad de la fórmula (I) o (II), Y representa un grupo opcionalmente sustituido seleccionado de: arilo, por ejemplo, fenilo o naftilo, heteroarilo, por ejemplo piridinilo, tiazolilo, tienilo, benzofuranilo o indolilo, y O-arilo, por ejemplo, O-fenilo.

En una modalidad adicional de la fórmula (I) o (II), Y es sustituido por uno o más grupos seleccionados de OR⁵, por ejemplo, OH o OCH₃, halógeno, por ejemplo F o Cl, arilo, por ejemplo, fenilo, halogenoalquilo de C₁₋₆, por ejemplo, CF₃ o CH₂CF₃, OCF₃, (R⁵)_pCN, por ejemplo, CN, (CH₂)_q-N(R⁵)-S(O)₂R⁸, por ejemplo, NHS(O)₂CH₃ y S(O)₂R⁹, por ejemplo, S(O)₂CH₃.

En una modalidad de la fórmula (I) o (II), cuando X representa A o A1, y A representa cicloalquilo, cicloalquenilo, arilo, heteroarilo o heterociclilo, y A1 representa -CH₂-O-(CH₂)_wN(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)-, -CH₂-(O)_p-(CH₂)_qC(O)NR⁵-, -CH₂-N(R⁵)C(O)N(R⁵)-, o -CH₂-C(O)N((CH₂)_wOH)-, y Y representa un anillo, por ejemplo, cuando X representa oxadiazolilo, tetrazolilo o pirazolilo y Y representa fenilo, piridinilo, o

tenilo, m es un entero seleccionado de 3 y 4 y n es un entero seleccionado de 0 y 1, por ejemplo, m es 4 y n es 0, o m es 3 y n es 1 ;

En una modalidad de la fórmula (I) o (II), R^1 se selecciona de: hidrógeno; y alquilo de C_{1-10} que puede ser opcionalmente sustituido por uno o
 5 más de: cicloalquilo, heterociclilo, arilo, heteroarilo, halogenoalquilo de C_{1-8} , halógeno, ciano, $-OR^4$, $-(CH_2)_nCOR^5$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^7$, y $-(NH)_mCONR^5R^7$. En otra modalidad de la fórmula (I) o (II), R^1 se selecciona de: hidrógeno; y alquilo de C_{1-10} que puede ser opcionalmente sustituido por uno o más de: halógeno, ciano, $-OR^4$, $-(CH_2)_nCOR^4$, $-CO_2R^4$, $-OCOR^4$, -
 10 $(CH_2)_nNR^5R^6$, y $-(NH)_mCONR^5R^6$. En una modalidad adicional de la fórmula (I) o (II), R^1 se selecciona de: hidrógeno y alquilo de C_{1-8} opcionalmente sustituido con $-OH$, por ejemplo, metilo, butilo o CH_2CH_2OH .

En una modalidad de la fórmula (I) o (II), R^1 se selecciona de: hidrógeno y alquilo de C_{1-8} , por ejemplo, metilo o butilo.

15 En una modalidad de la fórmula (I) o (II), R^2 se selecciona de: alquilo de C_{4-10} y alqueniilo de C_{2-10} , cada uno de los cuales puede ser opcionalmente sustituido por uno o más de cicloalquilo, heterociclilo, arilo, heteroarilo, halogenoalquilo de C_{1-8} , halógeno, ciano, $-OR^4$, $-(CH_2)_nCOR^5$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^6$, y $-(NH)_mCONR^5R^6$. En otra modalidad de la
 20 fórmula (I) o (II), R^2 puede representar alquilo de C_{4-10} que puede ser opcionalmente sustituido por uno o más de cicloalquilo, heterociclilo, arilo, heteroarilo, halogenoalquilo de C_{1-8} , halógeno, ciano, $-OR^4$, $-(CH_2)_nCOR^4$, $-CO_2R^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^6$, y $-(NH)_mCONR^5R^6$. En una modalidad

adicional, R^2 se selecciona de alquilo de C_{4-6} , por ejemplo, butilo o pentilo.

En una modalidad de la fórmula (I) o (II), R^2 representa alquilo de C_{1-10} que puede ser opcionalmente sustituido por uno o más de: cicloalquilo, heterociclilo, arilo, heteroarilo, halogenoalquilo de C_{1-6} , halógeno, $-CN$, $-OR^4$, $-(CH_2)_nCOR^5$, $-C(O)OR^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^6$, y $-(NH)_pCONR^5R^6$. En una modalidad adicional de la fórmula (I) o (II), R^2 se selecciona de alquilo de C_{3-6} , por ejemplo, butilo o pentilo.

En una modalidad de la fórmula (I) o (II), R^3 se selecciona de: alquilo de C_{1-6} fluorado. En otra modalidad de la fórmula (I) o (II), R^3 se selecciona de: alquilo de C_{1-4} fluorado, por ejemplo, CF_3 , CHF_2 o CF_2CF_3 .

En una modalidad de la fórmula (I) o (II), R^4 se selecciona de: hidrógeno, alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ heterociclilo, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo. En otra modalidad de la fórmula (I) o (II) R^4 se selecciona de: hidrógeno y alquilo de C_{1-4} .

En una modalidad de la fórmula (I) o (II), R^1 se selecciona de: hidrógeno y alquilo de C_{1-6} opcionalmente sustituido con $-OH$, R^2 se selecciona de: alquilo de C_{4-6} y R^3 se selecciona de: alquilo de C_{1-4} fluorado, con la condición de que cuando R^1 representa metilo y R^3 representa CF_3 , R^2 no puede ser i-butilo.

En una modalidad de la fórmula (I) o (II), R^5 es hidrógeno.

En una modalidad de la fórmula (I) o (II), R^7 se selecciona de: hidrógeno y alquilo de C_{1-4} .

En una modalidad adicional de la fórmula (I) o (II), siempre que cuando X es A1, A1 es -O- y Y es un anillo que es sustituido por arilo o heteroarilo, entonces m es un entero seleccionado de 3, 4 y 5.

En una modalidad de la fórmula (I) o (II), R¹ y R² son diferentes.

5 Cabe entender que la presente invención incluye cualquier combinación de modalidades particulares y cubre todas las combinaciones de sustituyentes particulares descritas anteriormente para compuestos de la fórmula (I) o (II).

Los compuestos particulares de la presente invención incluyen:

10 3-butil-1-metil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona,
1,3-dibutil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona,
3-butil-1-metil-8-(pentafluoroetil)-3,7-dihidro-1H-purino-2,6-diona,
8-(difluorometil)-1-metil-3-pentil-3,7-dihidro-1H-purino-2,6-diona,
3-pentil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona,
15 1-(2-hidroxietil)-3-pentil-8-(trifluorometil)-3,7-dihidro-1H-purino-
2,6-diona,

3-Butil-1-{3-[3-(fenilometil)-1,2,4-oxadiazol-5-il]propil}-8-
(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona,

Acido 4-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-
20 purin-1-il]butanoico,

3-Butil-1-[4-(3-fenilo-1,2,4-oxadiazol-5-il)butil]-8-(trifluorometil)-
3,7-dihidro-1H-purino-2,6-diona,

Acido 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-

purin-1-il]pentanoico,

3-Butil-1-{4-[3-(2-fluorofenil)-1,2,4-oxadiazol-5-il]butil}-8-

(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona,

3-Butil-1-{4-[3-(4-fluorofenilo)-1,2,4-oxadiazol-5-il]butil}-8-

5 (trifluorometil)-3,7-dihidro-1H-purino-2,6-diona,

3-Butil-1-{4-[3-(2-piridinil)-1,2,4-oxadiazol-5-il]butil}-8-

(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona,

3-Butil-8-(trifluorometil)-1-(3-{3-[(2,4,6-trifluorofenilo)metil]-1,2,4-

oxadiazol-5-il}propil)-3,7-dihidro-1H-purino-2,6-diona,

10 1-Metil-3-pentil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona.

La cantidad de un modulador de HM74A que se requiere para lograr el efecto biológico deseado, desde luego, dependerá de un número de factores, por ejemplo, el modo de administración y la condición clínica precisa del receptor. En general, la dosis diaria estará en el intervalo de 0.1 mg – 1

15 g/kg, típicamente 0.1 – 100 mg/kg. Una dosis intravenosa, por ejemplo, puede estar en el intervalo de 0.01 mg a 0.1 g/kg, típicamente 0.01 mg a 10 mg/kg, que se puede administrar convenientemente como una infusión de 0.1 µg a 1 mg, por minuto. Los fluidos de infusión adecuados para este propósito pueden contener, por ejemplo, de 0.01 µg a 0.1 mg, por mililitro. Las dosis unitarias

20 pueden contener, por ejemplo, de 0.01 µg a 1 g de un modulador de HM74A. Por lo tanto, las ampollitas para inyección pueden contener, por ejemplo, de 0.01 µg a 0.1 g y las formulaciones de dosis unitaria oralmente administrables, tales como tabletas o cápsulas, pueden contener, por ejemplo, de 0.1 mg a 1

g. Se indican o se esperan efectos no toxicológicos cuando un compuesto de la invención se administra en el intervalo de dosis antes mencionado.

Un compuesto de la presente invención se puede utilizar como el compuesto como tal en el tratamiento de una enfermedad en donde la sub-
5 activación del receptor de HM74A contribuye a la enfermedad o en donde la activación del receptor será benéfica, un ejemplo de esto es en donde un compuesto de la presente invención se presenta con un vehículo aceptable en forma de una formulación farmacéutica. El vehículo, desde luego, debe ser aceptable en el sentido de ser compatible con los otros ingredientes de la
10 formulación y no debe ser deletéreo para el receptor. El vehículo puede ser un sólido o un líquido, o ambos, y puede ser formulado con el modulador de HM74A como una formulación de dosis unitaria, por ejemplo, una tableta, que puede contener de 0.05% a 95% en peso del modulador de HM74A.

Las formulaciones incluyen aquellas adecuadas para
15 administración oral, rectal, tópica, bucal (v.gr., sub-lingual) y parenteral (v.gr., subcutánea, intramuscular, intradérmica o intravenosa).

También se provee de conformidad con la invención un procedimiento para la preparación de dicha composición farmacéutica que comprende mezclar los ingredientes.

20 Las formulaciones adecuadas para administración oral se puede presentar en unidades discretas, tales como cápsulas, cachets, trociscos o tabletas, cada una conteniendo una cantidad predeterminada de un modulador de HM74A; como un polvo o gránulos; como una solución o una

suspensión en un líquido acuoso o no acuoso; o como una emulsión de aceite en agua o agua en aceite. En general, las formulaciones se preparan mezclando en forma uniforme e íntima el modulador de HM74A activo con un vehículo líquido o sólido finamente dividido, o ambos, y después, si es necesario, configurar el producto. Por ejemplo, una tableta se puede preparar comprimiendo o modulando un polvo o gránulos del modulador de HM74A opcionalmente con uno o más ingredientes accesorios. Las tabletas comprimidas se pueden preparar comprimiendo, en una máquina adecuada, el compuesto en una forma de flujo libre, tal como un polvo o gránulos opcionalmente mezclado con un aglutinante, lubricante, diluyente inerte y/o agente(s) activos de superficie/dispersante(s). Las tabletas moduladas se pueden hacer modulando, en una máquina adecuada, el compuesto en polvo humedecido con un diluyente líquido inerte.

Las tabletas y cápsulas para administración oral pueden contener excipientes convencionales tales como agentes aglutinantes, por ejemplo, jarabe, acacia, gelatina, sorbitol, tragacanto, mucílago de almidón o polivinilpirrolidona; llenadores, por ejemplo, lactosa, celulosa microcristalina, azúcar, almidón de maíz, fosfato de calcio o sorbitol; lubricantes, por ejemplo, estearato de magnesio, ácido esteárico, talco, polietilenglicol o sílica; desintegrantes, por ejemplo, almidón de papa, croscarmelosa de sodio o glicolato de almidón de sodio; o agentes humectantes tales como laurilsulfato de sodio. Las tabletas pueden ser revestidas de conformidad con métodos bien conocidos en la técnica. Las preparaciones líquidas orales pueden ser,

por ejemplo, en forma de suspensiones acuosas u oleosas, soluciones, emulsiones, jarabes o elixires, o se puede presentar como un producto seco para constituirse con agua u otro vehículo adecuado antes de usarse. Dichas preparaciones líquidas pueden contener aditivos convencionales tales como

5 agentes de suspensión, por ejemplo, jarabe de sorbitol, metilcelulosa, jarabe de glucosa/azúcar, gelatina, hidroximetilcelulosa, carboximetilcelulosa, gel de estearato de aluminio o grasas comestibles hidrogenadas; agentes emulsionantes, por ejemplo, lecitina, monooleato de sorbitán o acacia; vehículos no acuosos (que pueden incluir aceites comestibles), por ejemplo,

10 aceite de almendra, aceite de coco fraccionado, ésteres oleosos, propilenglicol o alcohol etílico; o conservadores, por ejemplo, β -hidroxibenzoatos de metilo o propilo o ácido sórbico. Las preparaciones también pueden contener sales de regulador de pH, saborizante, colorante y/o agentes edulcorantes (v.gr., manitol) según sea apropiado.

15 Las formulaciones adecuadas para administración bucal (sublingual) incluyen trociscos que comprende un modulador de HM74A en una base saborizada, usualmente sacarosa y acacia o tragacanto, y pastillas, que comprende el modulador de HM74A en una base inerte tal como gelatina y glicerina o sacarosa y acacia.

20 Las formulaciones para administración oral se pueden formular adecuadamente para dar liberación controlada/extendida del compuesto activo.

Las formulaciones de la presente invención adecuadas para

administración parenteral convenientemente comprenden preparaciones acuosas estériles de un modulador de HM74A, la formulación puede ser isotónica con la sangre del receptor destinado. Estas preparaciones se podrían administrar por vía intravenosa, aunque la administración también se puede efectuar por medio de inyección subcutánea, intramuscular o intradérmica. Dichas preparaciones se pueden preparar convenientemente mezclando el modulador de HM74A con agua y haciendo la solución resultante estéril e isotónica con la sangre. Las composiciones inyectables de conformidad con la invención generalmente contendrán de 0.1 a 5% p/p del modulador de HM74A.

Por lo tanto, las formulaciones de la presente invención adecuadas para administración parenteral que comprenden un compuesto de conformidad con la invención se pueden formular para administración parenteral mediante inyección de bolo o infusión continua y se pueden presentar en forma de dosis unitaria, por ejemplo, como ampollitas, frascos, infusiones de volumen pequeño o jeringas prellenadas, o en contenedores de dosis múltiples con un conservador añadido. Las composiciones pueden adoptar formas tales como soluciones, suspensiones o emulsiones en vehículos acuosos o no acuosos, y pueden contener agentes formuladores tales como anti-oxidantes, reguladores de pH, agentes antimicrobianos y/o agentes ajustadores de toxicidad. Alternativamente, el ingrediente activo puede estar en forma de polvo para constitución con un vehículo adecuado, v.gr., agua estéril libre de pirógenos, antes de usarse. La presentación sólida

seca se puede preparar llenando con un polvo estéril de manera aséptica contenedores estériles individuales o llenando con una solución estéril de manera aséptica cada contenedor y secando por congelamiento.

Las formulaciones adecuadas para administración rectal se pueden presentar como supositorios de dosis unitaria. Estos se pueden preparar mezclando un modulador de HM74A con uno o más vehículos sólidos convencionales, por ejemplo, manteca de cacao o glicéridos y después configurando la mezcla resultante. Las formulaciones adecuadas para aplicación tópica a la piel pueden adoptar la forma de una pomada, crema, loción, pasta, gel, aspersión, aerosol o aceite. Los vehículos que se pueden usar incluyen vaselina, lanolina, polietilenglicoles, alcoholes, y combinaciones de dos o más de los mismos. El modulador de HM74A generalmente está presente a una concentración de 0.1 a 15% p/p de la composición, por ejemplo, de 0.5 a 2%.

Por administración tópica, como se usa aquí, los inventores de la presente incluyen administración por insuflación e inhalación. Ejemplos de varios tipos de preparación para administración tópica incluyen pomadas, cremas, lociones, polvos, pesarios, aspersiones, aerosoles, cápsulas o cartuchos para usarse en un inhalador o insuflador o gotas (v.gr., gotas para los ojos o nariz).

Las pomadas y cremas se pueden formular, por ejemplo, con una base acuosa u oleosa con la adición de agentes espesantes y/o gelificantes y/o solventes adecuados. Dichas bases por lo tanto pueden incluir,

por ejemplo, agua y/o un aceite tal como parafina líquida o aceite vegetal tal como aceite de araxis o aceite de ricino o un solvente tal como un polietilenglicol. Los agentes espesantes que se pueden usar incluyen parafina suave, estearato de aluminio, alcohol cetosteárfico, polietilenglicoles, cera microcristalina y cera de abeja.

Las lociones se pueden formular con una base acuosa u oleosa y en general también contienen uno o más agentes emulsionantes, agentes estabilizantes, agentes dispersantes, agentes de suspensión o agentes espesantes.

Los polvos para aplicación externa se pueden formar con la ayuda de cualquier base de polvo adecuada, por ejemplo, talco, lactosa o almidón. Las gotas se pueden formular con una base acuosa o no acuosa también que comprende uno o más agentes dispersantes, agentes solubilizantes o agentes de suspensión.

Las composiciones de aspersión se pueden formular, por ejemplo, como soluciones o suspensiones acuosas o como aerosoles suministrados desde paquetes presurizados, con el uso de un propelente adecuado, v.gr., diclorodifluorometano, triclorofluorometano, diclorotetrafluoroetano, 1,1,1,2,3,3,3-heptafluoropropano, 1,1,1,2-tetrafluoroetano, dióxido de carbono u otro gas adecuado.

Se pueden formular cápsulas y cartuchos para usarse en un inhalador o insuflador, de por ejemplo gelatina, que contengan una mezcla de polvo de un compuesto de la invención y una base de polvo adecuada tal

como lactosa o almidón.

Las composiciones farmacéuticas de conformidad con la invención también se pueden usar en combinación con otros agentes terapéuticos, por ejemplo, en combinación con otras clases de fármacos dislipidémicos (v.gr., estatinas, fibratos, resinas de ácido biliar o ácido nicotínico).

Los compuestos de la presente invención se pueden usar en combinación con uno o más de otros agentes terapéuticos, por ejemplo, en combinación con otras clases de fármacos dislipidémicos, v.gr., inhibidores de 3-hidroxi-3-metilglutaril-coenzima A reductasa (estatinas) o fibratos o resinas de unión de ácido biliar o ácido nicotínico. La invención por lo tanto provee, en un aspecto adicional, el uso de dicha combinación en el tratamiento de enfermedades en donde la sub-activación del receptor de HM74A contribuye a la enfermedad o en donde la activación del receptor será benéfica y el uso de un compuesto de la fórmula (I) o (II), o una sal farmacéuticamente aceptable, solvato o derivado farmacéuticamente aceptable del mismo en la fabricación de un medicamento para la terapia de combinación de trastornos del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteínaemia tales como dislipidemia diabética y dislipidemia mixta, insuficiencia cardíaca, hipercolesterolemia, enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis e hipertrigliceridemia, diabetes mellitus de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa u obesidad.

Cuando los compuestos de la presente invención se usan en

combinación con otros agentes terapéuticos, los compuestos se pueden administrar ya sea secuencialmente o simultáneamente por cualquier vía conveniente.

Las combinaciones referidas anteriormente convenientemente se pueden presentar para usarse en forma de una formulación farmacéutica y por lo tanto formulaciones farmacéuticas que comprenden una combinación como se definió antes de manera óptima junto con un vehículo o excipiente farmacéuticamente aceptable comprenden un aspecto adicional de la invención. Los componentes individuales de dichas combinaciones se pueden administrar ya sea secuencialmente o simultáneamente en formulaciones farmacéuticas separadas o combinadas.

Cuando se combinan en la misma formulación, se apreciará que los dos componentes deben ser estables y compatibles unos con otros y los otros componentes de la formulación y se pueden formular para administrarse. Cuando se formulan por separado, se pueden proveer en cualquier formulación conveniente, convenientemente de una manera como se conoce para dichos compuestos en la técnica.

Cuando en combinación con un segundo agente terapéutico activo contra la misma enfermedad, la dosis de cada componente puede diferir de aquella cuando el compuesto se usa solo. Las dosis apropiadas serán apreciadas fácilmente por los expertos en la técnica.

La invención por lo tanto provee, en un aspecto adicional, una combinación que comprende a compuesto de la fórmula (I) o un derivado

farmacéuticamente aceptable del mismo, junto con otro agente terapéuticamente activo.

La combinación anteriormente referida se puede presentar convenientemente para usarse en forma de una formulación farmacéutica y por lo tanto las formulaciones farmacéuticas que comprenden una combinación como se definió antes junto con un diluyente, excipiente o vehículo farmacéuticamente aceptable del mismo representan un aspecto adicional de la invención. Por ejemplo, una formulación farmacéutica que comprende a compuesto o derivado farmacéuticamente aceptable del mismo y uno o más diluyentes, excipientes o vehículos farmacéuticamente aceptables.

Purificación general y métodos analíticos

Los espectros de masa (EM) se registraron en un espectrómetro de masa Fisons VG Platform usando modos de ionización positiva de electroaspersión [(ES+ve para dar iones moleculares MH^+ y $M(NH_4)^+$] o ionización negativa de electroaspersión [(ES-ve para dar iones moleculares $(M-H)^-$].

Los espectros de 1H RMN se registraron usando un espectrómetro Bruker DPX 400 MHz usando tetrametilsilano como el estándar externo.

Cromatografía Biotage™ se refiere a una purificación llevada a cabo usando equipo vendido por Dyax Corporation (ya sea Flash 40i o Flash 150i) y

cartuchos pre-empacados con KPSil.

La autopreparación dirigida a la masa se refiere a métodos en donde el material se purificó por cromatografía de líquidos de alto rendimiento en una columna HPLCABZ+ 5 μm (5 cm x 10 mm de d.i.) con 0.1 % de HCO₂H en agua y 95% de MeCN, 5% de agua (HCO₂H al 0.5%) utilizando las siguientes condiciones de elución por gradientes: 0-1.0 minutos 5% de B, 1.0-8.0 minutos 5→30% de B, 8.0-8.9 minutos 30% de B, 8.9-9.0 minutos 30→95% de B, 9.0-9.9 minutos 95% de B, 9.9-10 minutos 95→0% de B a una velocidad de flujo de 8 ml minutos⁻¹ (Sistema 2). El recolector de fracciones Gilson 202 fue activado por un espectrómetro de masa de plataforma VG al detectar la masa de interés.

CLAR preparativa. se refiere a métodos en donde el material fue purificado por cromatografía de líquidos de alto rendimiento en una columna HPLCABZ+ 5 μm (10 cm x 21.2 mm d.i.) con 0.1% de HCO₂H en agua (A) y MeCN (HCO₂H al 0.5%) (B) utilizando las condiciones de elución por gradientes genéricas expresadas como gradiente de "x a y" con un sistema de gradientes como sigue: 0-1.45 minutos x% de B, 1.45-20 minutos x→y% de B, 20-24 minutos y→95% de B, 24-30 minutos 95% de B, 32-34 minutos 95→x% de B a una velocidad de flujo de 8 ml minutos⁻¹. El recolector de fracciones Gilson 233 fue activado por UV (254 nm).

La SPE (extracción de fase sólida) se refiere al uso de cartuchos vendidos por International Sorbent Technology Ltd.

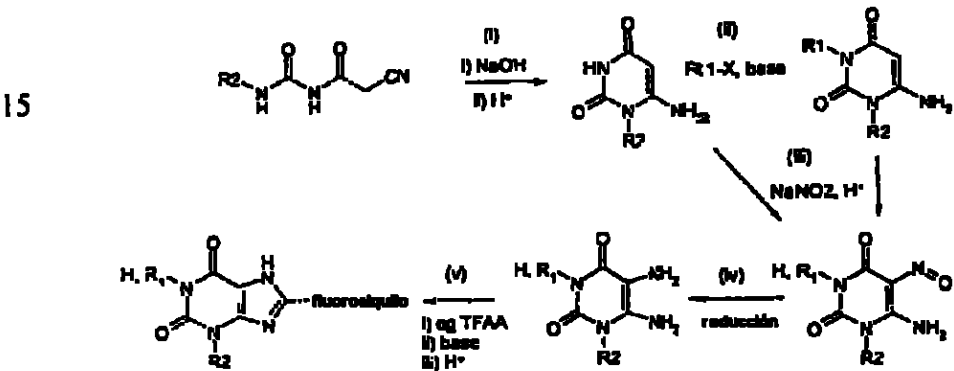
Strata Phenyl SPE se refiere al uso de cartuchos vendidos por

Phenomenex. El compuesto se cargó en un cartucho previamente acondicionado con MeCN y equilibrado con 5% de MeCN en agua. El compuesto se eluyó con HCO₂H al 0.1 % en agua y MeCN (HCO₂H al 0.5%) en un gradiente adecuado en un Combiflash Optix 10.

5 Los compuestos de la presente invención y derivados farmacéuticamente aceptables de los mismos se pueden preparar por la metodología descrita más adelante, que constituye un aspecto de esta invención.

10 Procedimiento A

Un procedimiento de conformidad con la invención para preparar un compuesto de la fórmula (I) o fórmula (II) en la cual R¹ es H o alquilo en donde R¹ y R² pueden ser los mismos o diferentes, comprende:



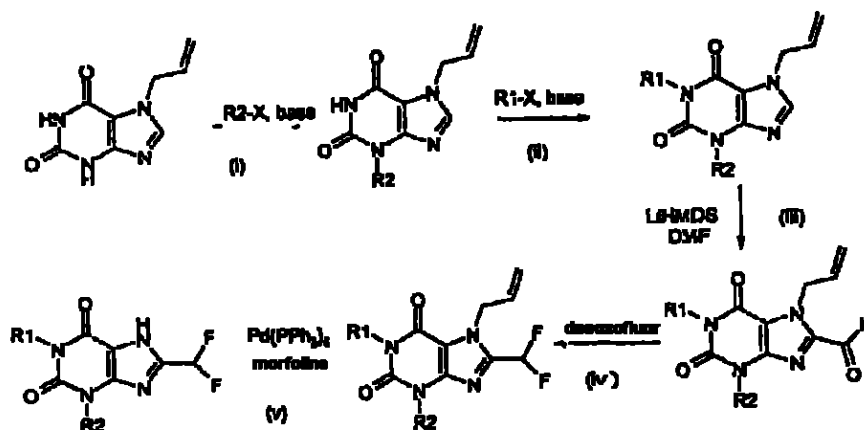
- 20
- i) Formación de pirimidinodiona
 - ii) Alquilación
 - iii) Nitrosación
 - iv) Reducción

v) Formación de xantina

Procedimiento B

Un procedimiento de conformidad con la invención para preparar un compuesto de la fórmula (I) o fórmula (II) en la cual R^1 y R^2 pueden ser los mismos o diferentes, comprende:

10



15

i) Alquilación selectiva en N3

ii) Alquilación en N1

iii) Formilación de C8

iv) Fluoración de carbonilo

v) Desprotección mediada por paladio

Cuando se desea o es necesario, como una etapa final en cualquiera de los procedimientos sintéticos anteriores, un compuesto resultante de la fórmula (I) o (II) se puede convertir en una forma de sal farmacéuticamente aceptable o viceversa o convierte una forma de sal en otra forma de sal farmacéuticamente aceptable.

20

Abreviaturas

	AcOH	Ácido acético
	br	Banda ancha (RMN)
	CDI	Carbonildiimidazol
5	D	Doblete (RMN)
	DBAD	Azodicarboxilato de di-t-butilo
	DCM	Diclorometano
	DIPEA	Diisopropiletilamina
	DMSO	Sulfóxido de dimetilo
10	DMF	N,N-Dimetilformamida
	EtOAc	Acetato de etilo
	EtOH	Etanol
	hr	Hora(s)
	IPA	alcohol isopropílico
15	M	Multiplete (RMN)
	MDAP	Autopreparación dirigida a masa
	MeCN	Acetonitrilo
	MeOH	Metanol
	Min	Minuto(s)
20	NCS	N-Clorosuccinimida
	PFPa	Anhídrido pentafluoropropiónico
	q	Cuadriplete (RMN)
	ta	Temperatura ambiente

	TR	Tiempo de retención
	S	Singulete (RMN)
	SPE	Cartucho de extracción de fase sólida
	t	Triplete (RMN)
5	TFA	Acido trifluoroacético
	TFAA	Anhídrido de ácido trifluoroacético
	THF	Tetrahidrofurano
	DMEM	medio de Eagle modificado por Dulbecco
	HEPES	ácido 4-(2-hidroxietil)piperazin-1 -etansulfónico
10	LIHMDS	hexametildisililamida de litio

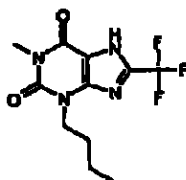
Los siguientes ejemplos no limitantes ilustran la presente invención:

EJEMPLOS DE SINTESIS

15

EJEMPLO 1

3-Butil-1-metil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona



20

Una mezcla púrpura de 5,6-diamino-1-butil-3-metil-2,4(1H,3H)-pirimidinodiona (European Journal of Medicinal Chemistry (1990), 25(8), 653-8) (290 mg) y TFAA (6 ml) se calentó a reflujo durante 1 hora, después se concentró bajo vacio. El residuo resultante se trató con NaOH 2N (ac) (7 ml) y

la mezcla se calentó a reflujo durante 30 minutos. Después de enfriar a temperatura ambiente, la mezcla se acidificó a aproximadamente pH 4 con HCl 3M (ac). El precipitado se recogió por filtración y se lavó con una solución de agua:EtOH (2:1) después agua:EtOH (1:1). El sólido color beige resultante se secó bajo vacío (100 mg, 25%).

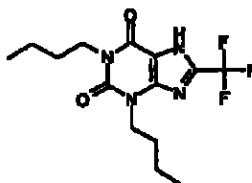
RMN; δ_H (400 MHz, d^6 -DMSO) 0.90 (t, 3H, $J=7.5$ Hz), 1.25-1.36 (m, 2H), 1.59-1.69 (m, 2H), 3.25 (s, 3H), 3.97 (t, 2H, $J=7$ Hz),

No se observó NH a δ_H 15; m/z 291.2 [MH^+].

10

EJEMPLO 2

1,2-Dibutil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona



15

Una mezcla púrpura de 5,6-diamino-1,3-dibutil-2,4(1H,3H)-pirimidinodiona (Journal of Medicinal Chemistry (1991), 34(1), 466-9) (207 mg) y TFAA (4 ml) se calentó a reflujo durante 1 hora, después se concentró bajo vacío. El residuo resultante se trató con NaOH 2N (ac) (5 ml) y la mezcla se calentó a reflujo durante 30 minutos. Después de enfriar a temperatura ambiente, la mezcla se acidificó a aproximadamente pH 4 con HCl 3M (ac). El precipitado se recogió por filtración y se purificó por recristalización a partir de una solución de agua:EtOH (2:1). El sólido café pálido/anaranjado se secó bajo vacío a 50°C durante 48 horas para proveer el compuesto del título como

20

un sólido color beige (35 mg, 13%).

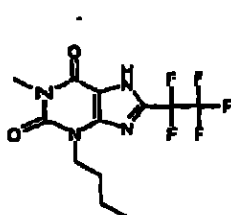
δ_H (400 MHz, d^6 -DMSO) 0.90 (t, 6H, $J=7$ Hz), 1.26-1.34 (m, 4H), 1.48-1.68 (traslapando m, 4H), 3.89 (t, 2H, $J=7$ Hz), 3.97 (t, 2H, $J=7$ Hz),

NH no se observó a δ_H 15; m/z 333.2 $[MH^+]$.

5

EJEMPLO 3

3-Butil-1-metil-8-(pentafluoroetil)-3,7-dihidro-1H-purino-2,6-diona



10

Una mezcla púrpura de 5,6-diamino-1-butil-3-metil-2,4(1H,3H)-pirimidinodiona (European Journal of Medicinal Chemistry (1990), 25(8), 653-8) (300 mg, 1.41 mmoles) y anhídrido pentafluoropropiónico (PFPA) (6 ml) se calentó a 60°C durante 1 hora, después se concentró bajo vacío. El residuo resultante se trató con NaOH 2N (ac) (7 ml) y la mezcla se calentó a 90°C durante 30 minutos. Después de enfriar hasta temperatura ambiente, la mezcla se acidificó a aproximadamente pH 4 con HCl 3M (ac). El precipitado se recogió por filtración, se lavó con agua y se secó bajo vacío para dar el compuesto del título como un sólido esponjoso verde pálido/café (163 mg, 34%).

15

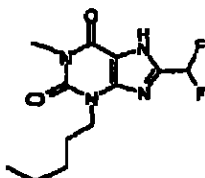
20

δ_H (400 MHz, d^6 -DMSO) 0.89 (t, 3H, $J=7.5$ Hz), 1.24-1.35 (m, 2H), 1.60-1.69 (m, 2H), 3.25 (s, 3H), 3.98 (t, 2H, $J=7$ Hz),

NH no se observó a δ_H 15; m/z : 358.4 $[MNH_4^+]$.

EJEMPLO 4**8-(Difluorometil)-1-metil-3-pentil-3,7-dihidro-1H-purino-2,6-diona**

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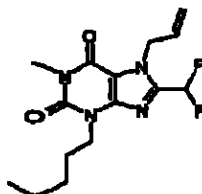


Una solución de 8-(difluorometil)-1-metil-3-pentil-7-(2-propen-1-il)-3,7-dihidro-1H-purino-2,6-diona en THF anhidro (3 ml) y DMSO anhidro (0.3 ml) se desgasificó bajo un vacío moderado y se introdujo nitrógeno. Esto se repitió dos veces. Se añadió Pd(PPh₃)₄ (74 mg, 0.064 mmoles) y la mezcla se desgasificó una vez más. Se añadió morfolina (374 μ l, 4.3 mmoles) y se dejó agitar a temperatura ambiente durante 4 horas. La mezcla se dividió entre HCl 2M (ac) y EtOAc. La capa orgánica se separó, se lavó con salmuera, se secó (MgSO₄) y se concentró. El residuo se recogió en MeOH y se hizo pasar por una columna de amino-propilo-SPE (5 g), eluyendo con MeOH seguido por 5% de AcOH/MeOH. La fracción de producto se concentró para dar el compuesto del título como un sólido blanquecino (98 mg, 80%).

δ_H (400 MHz, d⁶-DMSO) 0.85 (t, 3H, J=7Hz), 1.23-1.35 (m, 4H), 1.61-1.71 (m, 2H), 3.25 (s, 3H), 3.96 (t, 2H, J=7.5Hz), 7.12 (t, 1H, J=53Hz), 14.59 (br. s, 1H);

m/z 287.2 [MH⁺].

8-(Difluorometil)-1-metil-3-pentil-7-(2-propen-1-il)-3,7-dihidro-1H-purino-2,6-diona



5

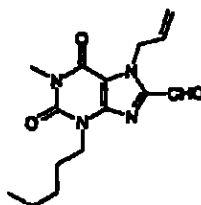
1-Metil-2,6-dioxo-3-pentil-7-(2-propen-1-il)-2,3,6,7-tetrahidro-1H-purino-8-carbaldehído (133 mg, 0.44 mmoles) se añadió a un matraz previamente secado bajo nitrógeno. DCM anhidro (3 ml) se añadió seguido por desoxofluor (161 μ l, 0.87 mmoles) y finalmente EtOH (1 gota, aproximadamente 0.09 mmoles). La mezcla se dejó agitar a temperatura ambiente durante 4 horas, después se dividió entre NaHCO_3 (ac) sat. y DCM. La capa orgánica se separó, se lavó con salmuera, se secó (MgSO_4) y se concentró para dar el compuesto del título como un aceite amarillo (145 mg).

10

m/z 327.4 $[\text{MH}^+]$.

15

1-Metil-2,6-dioxo-3-pentil-7-(2-propen-1-il)-2,3,6,7-tetrahidro-1H-purino-8-carbaldehído



20

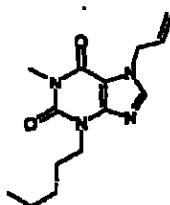
A una solución de 1-metil-3-pentil-7-(2-propen-1-il)-3,7-dihidro-1H-purino-2,6-diona (1.05 g, 3.8 mmoles) en THF (15 ml) a -78°C se añadió

1A

hexametildisililazida de litio (solución en hexano 1M, 4 ml, 4 mmoles) durante 10 minutos. La solución se agitó durante 0.5 hr, después se añadió DMF (0.5 ml, 6.5 mmoles) y la solución se agitó durante 0.5 hr adicionales a -78°C , después de lo cual se dejó calentar a temperatura ambiente durante 2 hr. La
5 reacción se extinguió con solución de HCl 2N, después se separó entre EtOAc/salmuera. La cromatografía sobre sílice (elución por gradientes con DCM a 5:1 DCM/EtOAc) dio el compuesto del título (0.35 g, 30%).

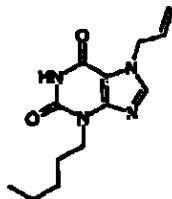
m/z 305 $[\text{MH}^+]$.

10 1-Metil-3-pentil-7-(2-propen-1-il)-3,7-dihidro-1H-purino-2,6-diona



15 A una solución de 3-pentil-7-(2-propen-1-il)-3,7-dihidro-1H-purino-2,6-diona (0.20 g, 0.76 mmoles) y yoduro de metilo (0.5 ml, 4.9 mmoles) en DMF (5 ml) se añadió carbonato de potasio (0.4 g, 2.9 mmoles) y la mezcla se calentó a 50°C durante 3 hr, se enfrió y la mezcla se separó entre EtOAc/salmuera. Los compuestos orgánicos se aislaron, se secaron
20 (MgSO_4) y se concentraron para dar el producto requerido (0.21 g, 100%).

m/z 277 $[\text{MH}^+]$.

3-Pentil-7-(2-propen-1-il)-3,7-dihidro-1H-purino-2,6-diona

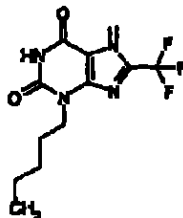
5

A una solución de 7-(2-propen-1-il)-3,7-dihidro-1H-purino-2,6-diona (0.61 g, 3.2 mmoles) y yoduro de pentilo (0.64 g, 3.2 mmoles) en DMF (7 ml) se añadió carbonato de sodio (0.60 g, 5.7 mmoles) y la mezcla se calentó a 50°C durante 18 hr. Después de enfriarse, la mezcla se separó entre

10 EtOAc/salmuera. Los compuestos orgánicos se aislaron, se secaron (MgSO₄) y se concentraron para dar el producto crudo que se purificó por cromatografía sobre sílice (elución por gradientes con DCM a 5:1 DCM/EtOAc) para proveer el compuesto del título (0.47 g, 56%).

m/z 263 [MH⁺].

15

EJEMPLO 5**3-Pentil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona**

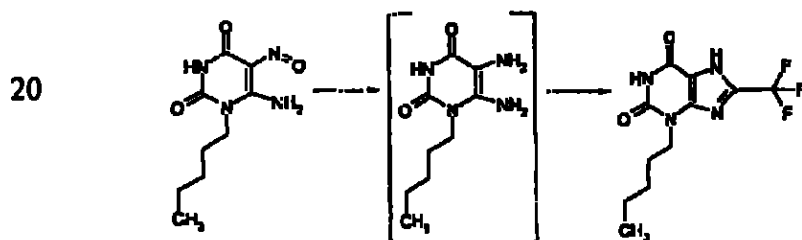
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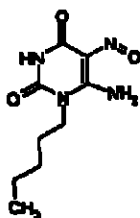
Una mezcla de 6-amino-5-nitroso-1-pentil-2,4(1H,3H)-pirimidinodiona (500 mg, 2.21 mmoles), 10% de Pd/C (100 mg) y EtOH (100

ml) se agitó vigorosamente bajo una atmósfera de hidrógeno durante 4 horas. La mezcla se filtró a través de Celite y el filtrado se concentró bajo vacío, dando 5,6-diamino-1-pentil-2,4(1*H*,3*H*)-pirimidinodiona como un sólido verde pálido (62 mg) y como una mezcla con un producto desconocido (2:1). Se
 5 añadió anhídrido trifluoroacético (5 ml) y se calentó a reflujo durante 1 hora después se concentró bajo vacío. NaOH 2M (ac) (15 ml) se añadió al residuo crudo y se calentó a 100°C durante 30 minutos. Después de dejarla enfriar hasta temperatura ambiente, la mezcla se acidificó a aproximadamente pH 5 con HCl 2M (ac). El precipitado resultante se recogió por filtración y después
 10 se recogió en MeOH con calentamiento y después se hizo pasar por una columna de aminopropilo (5 g), eluyendo con 2% de AcOH/MeOH, 5% de AcOH/MeOH, 10% de AcOH/MeOH y 15% de AcOH/MeOH. La fracción de producto se concentró, dando el compuesto del título como un sólido blanquecino (52 mg).

15 RMN; (400 MHz, d^6 -DMSO) δ_H 0.85 (t, 3H, $J=7$ Hz), 1.22-1.31 (m, 4H), 1.61-1.66 (quintuplete, 2H, $J=7$ Hz), 3.89 (t, 2H, $J=7$ Hz), 11.39 (s, 1 H), 15.3 (br. s, 1 H).

m/z 291.2 [MH^+].



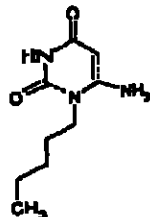
6-Amino-5-nitroso-1-pentil-2,4(1*H*,3*H*)-pirimidinodiona

5

Una mezcla de 6-amino-1-pentil-2,4(1*H*,3*H*)-pirimidinodiona (3.0 g, 15.2 mmoles), AcOH (6 ml) y HCl 6M (ac) se enfrió a 0°C, después se trató gota a gota con una solución de NaNO₂ (1.2 g, 16.7 mmoles) en agua (4 ml). La mezcla se agitó durante 45 minutos y se añadió agua (aproximadamente 200 ml). El sólido púrpura resultante se filtró, se lavó con agua (aproximadamente 200 ml) y se secó bajo alto vacío a 60°C durante 18 horas. Se dio el compuesto del título como un sólido púrpura (2.08 g, 60%).

m/z 227.3 [MH⁺].

15

6-Amino-1-pentil-2,4(1*H*,3*H*)-pirimidinodiona

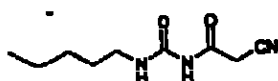
20

Una mezcla de 2-ciano-*N*-[(pentilamino)carbonilo]acetamida (17.9 g, 0.091 moles), EtOH (70 ml) y agua (40 ml) se calentó a 85°C y después se añadió 10% de NaOH (ac) (10 ml) se añadió gota a gota. La reacción se agitó durante 1 hora antes de dejarse enfriar a temperatura

ambiente, después se diluyó con agua y se acidificó a aproximadamente pH 6 usando HCl 2M (ac). La mezcla se enfrió (baño de hielo), después se filtró y el sólido resultante se lavó con agua, después se secó bajo vacío a 40°C. Esto dio el compuesto del título como un sólido blanco (11.0 g, 61 %).

5 m/z 198.2 [MH⁺].

2-Ciano-N-[(pentilamino)carbonil]acetamida



10 Una mezcla de pentilurea (16.4 g, 0.13 moles), ácido cianoacético (11.8 g, 0.14 moles) y anhídrido acético (30 ml) se calentó a 80°C durante 2 horas. La mezcla se dejó enfriar a temperatura ambiente y se agitó con Et₂O (aproximadamente 100 ml) durante 1 hora. La mezcla se enfrió a 0°C (baño de hielo) y el sólido se recogió por filtración dando el compuesto

15 del título como un sólido blanco (17.9 g, 72%).

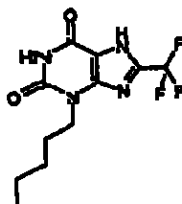
m/z 196.1 [MH⁺].

EJEMPL 5(b)

119

3-Pentil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona (método alternativo)

5



A una suspensión de 6-amino-5-nitroso-1-pentil-2,4(1H,3H)-
pirimidinodiona (0.227 g, 1 mmol) en diclorometano anhidro (5 ml) se añadió
anhídrido trifluoroacético (0.35 ml, 2.5 mmoles). La suspensión amarillo pálido
10 resultante se agitó durante 40 minutos a temperatura ambiente y después la
solución se concentró bajo vacío.

El residuo se disolvió en dioxano (25 ml) y se añadió a 10% de
paladio sobre carbón (0.5 g) y se hidrógeno durante 1 hr. El catalizador se
filtró, se lavó con dioxano y el filtrado se concentró bajo vacío. Algo del
15 material de partida permaneció por lo que el paso de hidrogenación se repitió.
Después de 1.5 hr adicionales, el catalizador se filtró, se lavó con dioxano y el
filtrado se concentró bajo vacío.

El residuo se disolvió en tetrahidrofurano (10 ml) y se añadió
NaOH 2N (5 ml). La solución se agitó a 60°C durante 2 hr. La mezcla de
20 reacción cruda se diluyó con acetato de etilo y agua y después se acidificó
con HCl 2N. La fase orgánica se lavó con salmuera, se secó (MgSO₄) y se
concentró bajo vacío para dar el compuesto del título como un sólido
anaranjado (140 mg, 48%).

LC/EM: m/z 291 [MH]⁺, TR 3.00 min

120

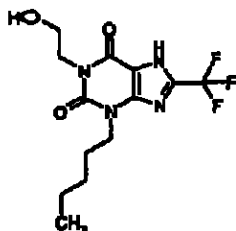
¹H RMN (DMSO-d₆) δ_H 0.85 (3H, t, J = 7Hz), 1.28 (4H, m), 1.64 (2H, m), 3.88 (2H, t, J = 7Hz), 11.22 (1 H, br s).

EJEMPLO 6

121

1-(2-Hidroxietil)-3-pentil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona

5



Una solución de 6-amino-1-pentil-3-[2-(tetrahidro-2H-piran-2-iloxi)etil]-2,4(1H,3H)-pirimidinodiona (970 mg) en AcOH (10 ml) se trató con HCl 6M (ac) (8 ml) y después se añadió una solución de NaNO₂ (247 mg) en

10 agua (3 ml). Después de 15 minutos, la mezcla se dividió entre agua y EtOAc. La capa orgánica se separó y la capa acuosa se extrajo con EtOAc (x7). Los extractos combinados se lavaron con salmuera, se secaron (MgSO₄) y se concentraron, dando un aceite púrpura (446 mg). Una suspensión de este aceite en DCM (12 ml) se trató con la adición gota a gota de TFAA (12 ml).

15 Después de 10 minutos, la mezcla se concentró dando un aceite amarillo/café (798 mg). Una porción de este material (150 mg) se hizo reaccionar bajo una atmósfera de hidrógeno en THF (15 ml) durante 2.5 horas. La mezcla se filtró a través de celite y se lavó completamente con THF. El filtrado se concentró y se trató una vez más con TFAA (5 ml) y después se concentró bajo vacío.

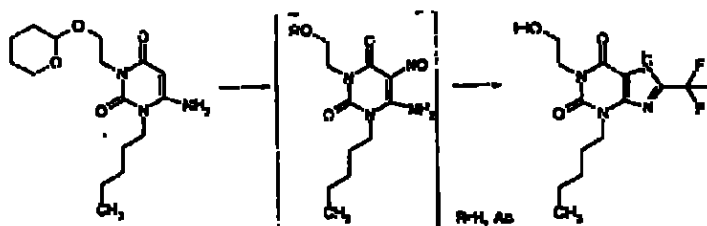
20 NaOH 2M (ac) (4 ml) se añadió al residuo y la mezcla se calentó a 50°C durante 30 minutos. Después de enfriamiento, la mezcla se acidificó a aproximadamente pH 5 y el producto se extrajo con EtOAc. La capa orgánica se separó, se lavó con salmuera, se secó (MgSO₄) y se concentró. El residuo

122

resultante se hizo pasar por una CLAR autopreparativa para dar el compuesto del título (32 mg, 17%).

RMN; (400 MHz, d6-DMSO) δ_H 0.85 (t, 3H, J=7Hz), 1.23-1.36 (m, 4H), 1.62-1.70 (m, 2H), 3.52 (t, 2H, J=6.5Hz), 3.97 (m, 4H), 15.5 (br. S, 1 H).

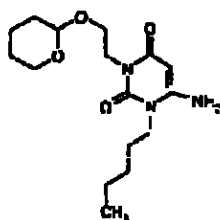
5 m/z 335.3 [MH⁺].



10

6-Amino-1-pentil-3-[2-(tetrahidro-2H-piran-2-iloxi)etil]-2,4(1H,3H)-pirimidinodiona

15



Una mezcla de 6-amino-1-pentil-2,4(1H,3H)-pirimidinodiona (300 mg, 1.5 mmoles), Cs₂CO₃ (595 mg, 1.8 mmoles), 2-(2-bromoetoxi)tetrahidro-2H-piran (276 μ l, 1.8 mmoles) y DMF anhidro (5 ml) se calentó a 40°C durante 18 horas. La mezcla se dividió entre agua y EtOAc. La capa orgánica se separó, se lavó con salmuera, se secó (MgSO₄) y se concentró, dando una goma café pálido (198 mg, 40%).

m/z 326.3 [MH⁺].

EJEMPL 7(a)

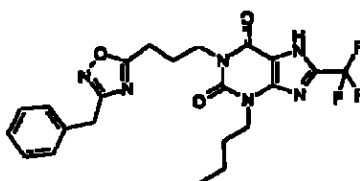
3-Butil-1-{3-[3-(fenilmetil)-1,2,4-oxadiazol-5-il]propil}-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona

5

EJEMPLO 7(b)

Acido 4-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]butanoico

a) **3-Butil-1-{3-[3-(fenilmetil)-1,2,4-oxadiazol-5-il]propil}-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona**



Acido 4-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]butanoico (0.181 g, 0.5 mmoles) y CDI (0.089 g, 0.55 mmoles) se disolvieron en dimetilformamida anhidra (6 ml). Después de agitar la solución bajo nitrógeno durante 3 hr a temperatura ambiente, bencilamidoxima (0.083 g, 0.55 mmoles) se añadió y la solución se calentó a 90°C durante 20 hr, después a 110°C durante 4 hr. La mezcla de reacción se concentró bajo vacío a un aceite amarillo. Este se purificó en un Teledyne Isco CombiFlash Companion® usando un cartucho de sílice RediSep de 40 g (elución por gradientes con cloroformo/etanol 0-15%). Las fracciones apropiadas se evaporaron bajo vacío, después se secó por congelamiento a partir de

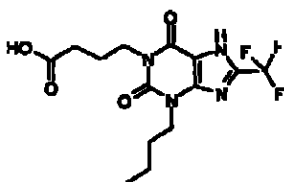
dioxano para dar el compuesto del título como un liofilato blanco (142 mg, 59%)

LC/EM: m/z 477 [MH]⁺, TR 3.57 min

¹H RMN (CDCl₃) δ_H 0.98 (3H, t, J = 7Hz), 1.41 (2H, m), 1.76 (2H, m), 2.23 (2H, m), 2.95 (2H, t, J = 8Hz), 4.00 (2H, s), 4.14 (2H, t, J = 8Hz), 4.22 (2H, t, J = 7Hz), 7.27 (5H, s)

b) Acido 4-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]butanoico

10



Se añadió diclorometano anhidro (50 ml) a 4-(4-amino-3-butyl-5-nitroso-2,6-dioxo-3,6-dihidro-1(2H)-pirimidinil)butanoato de etilo (3.26 g, 10 mmoles), seguido por anhídrido trifluoroacético (3.53 ml, 25 mmoles). La solución amarilla se agitó a temperatura ambiente bajo una atmósfera de nitrógeno durante 15 minutos y después se concentró bajo vacío. El residuo se disolvió en dioxano (150 ml) y se añadió a 10% de paladio sobre carbón (0.5 g) y se hidrógeno durante 2 hr. El catalizador se filtró, se lavó con dioxano y el filtrado se concentró bajo vacío.

20

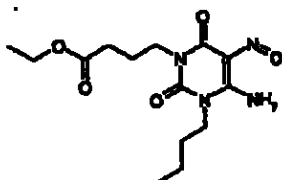
El residuo se disolvió en tetrahidrofurano (100 ml) y NaOH 2N (50 ml) se añadió. La solución se agitó a 60°C durante 1.5 hr, se enfrió y se diluyó con acetato de etilo (100 ml) y agua (30 ml). La fase orgánica se extrajo

con agua (2x 30 ml). Los extractos acuosos combinados se acidificaron a pH 4 con HCl 2N. El sólido resultante se filtró y se lavó con agua para dar el compuesto del título como un sólido color crema pálido 2.17 g (60%).

LC/EM: m/z 363 [MH]⁺, TR 2.84 min

5 ¹H RMN (DMSO-d₆) δ_H 0.90 (3H, t, J = 7Hz), 1.31 (2H, m), 1.64 (2H, m), 1.80 (2H, m), 2.24 (2H, t, J = 7Hz), 3.89 (2H, t, J = 7Hz), 3.95 (4H, m), 12.01, (1 H, br s), 15.36, (1 H, br s)

10 c) 4-(4-Amino-3-butil-5-nitroso-2,6-dioxo-3,6-dihidro-1(2H)-pirimidinil)butanoato de etilo



15 4-(4-Amino-3-butil-2,6-dioxo-3,6-dihidro-1(2H)-pirimidinil)-
butanoato de etilo (8.5 g, 29.8 mmoles) se disolvió en una mezcla de ácido
acético glacial (45 ml) y ácido clorhídrico 6M (24 ml). La solución se enfrió a –
1°C y nitrito de sodio (2.26 g, 32.7 mmoles) en agua (20 ml) se añadió gota a
gota. Después de que se completó la adición, se removió el baño de hielo y la
solución púrpura se agitó durante 1 hr. La solución se diluyó con agua (140
20 ml) y el producto, que se separó después de unos minutos, se filtró, se lavó
con agua (350 ml en total) y se secó bajo vacío a 50°C para dar el compuesto
del título como un sólido rosa brillante (6.61 g). En reposo, se aisló una
cosecha adicional (0.53 g), después las soluciones madre se basificaron a pH

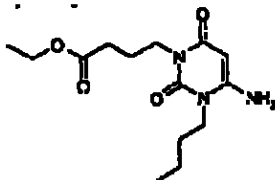
6 con hidróxido de sodio 2N y se extrajeron en acetato de etilo (2x 250 ml). Los extractos combinados se concentraron bajo vacío para dar una tercera cosecha del compuesto del título (1.23 g). Rendimiento total 8.37 g 86%.

LC/EM: m/z 327 [MH]⁺, TR 2.58 min

5

d) 4-(4-Amino-3-butil-2,6-dioxo-3,6-dihidro-1(2H)-pirimidinil)

butanoato de etilo



10

6-Amino-1-butyl-2,4(1*H*,3*H*)-pirimidinodiona (5.9 g, 32.2 mmoles) y carbonato de cesio (11.54 g, 35.4 mmoles) se añadieron a dimetilformamida anhidra bajo una atmósfera de nitrógeno. Se añadió 4-bromobutirato de etilo (5.07 ml, 35.4 mmoles) y la mezcla se agitó a temperatura ambiente durante 15 22 hr. La mezcla de reacción se diluyó con acetato de etilo (350 ml) y se lavó con solución de cloruro de sodio saturada (2 x 50 ml). Las fases acuosas combinadas se extrajeron otra vez con acetato de etilo. Las fases orgánicas combinadas se lavaron con solución de cloruro de sodio saturada, se secaron (MgSO₄) y se concentraron bajo vacío. El producto crudo se purificó en un 20 Teledyne Isco CombiFlash® Companion® usando un cartucho de sílice RediSep de 80 g (elución por gradientes usando cloroformo/etanol 0-40%). Las fracciones apropiadas se evaporaron bajo vacío para dar el compuesto del título como un aceite amarillo que se cristalizó en reposo (5.36 g, 56%)

LC/EM: m/z 298 [MH]⁺, TR 2.49 min

127

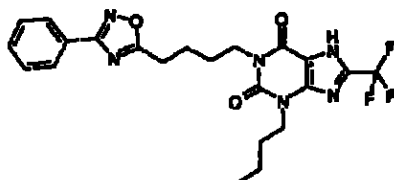
EJEMPLO 8 (a)

3-Butil-1-[4-(3-fenil-1,2,4-oxadiazol-5-il)butil]-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona

EJEMPLO 8 (b)

Acido 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]pentanoico

a) 3-Butil-1-[4-(3-fenil-1,2,4-oxadiazol-5-il)butil]-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona



Una mezcla de 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]pentanoato de metilo (0.156 g, 0.4 mmoles), benzamidoxima (60 mg, 0.44 mmoles), solución de NaOEt al 21% en EtOH (0.22 ml, 0.6 mmoles) y EtOH anhidro (1 ml) se calentó en el microondas a 140°C durante 10 minutos. La mezcla se diluyó con EtOAc (30 ml) y se lavó con agua (20 ml), HCl 2N (20 ml) y salmuera (5 ml). La capa orgánica se secó (Na₂SO₄) y se concentró bajo vacío a un aceite amarillo y después se secó por congelamiento a partir de dioxano para dar el compuesto del título como

128

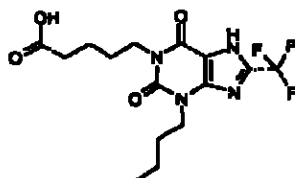
un liofilato color crema (95 mg, 50%)

LC/EM: m/z 476 $[MH]^+$, TR 3.73 min

1H RMN ($CDCl_3$) δ_H 0.97 (3H, t, $J = 7$ Hz), 1.41 (2H, m), 1.77 (2H, m), 1.87 (2H, m), 1.97 (2H, m), 3.02 (2H, t, $J = 7$ Hz), 4.17 (4H, m), 7.47 (3H, m), 8.04 (2H, m)

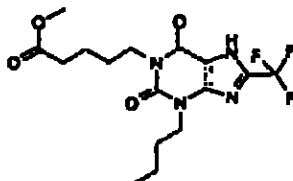
b) Acido 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]pentanoico

10



y 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]pentanoato de metilo

15



Se añadió diclorometano anhidro (70 ml) a 4-(4-amino-3-butil-5-nitroso-2,6-dioxo-3,6-dihidro-1(2H)-pirimidinil)pentanoato de metilo (5.06 g, 15.5 mmoles), seguido por anhídrido trifluoroacético (22 ml, 10 equiv.). La solución amarilla se agitó a temperatura ambiente bajo una atmósfera de nitrógeno durante 25 minutos y después se concentró bajo vacío. El residuo se disolvió en dioxano (250 ml) y se añadió a 10% de paladio sobre carbón

(0.85 g) y se hidrógeno durante 2.25 hr. El catalizador se filtró, se lavó con dioxano y el filtrado se concentró bajo vacío. El residuo se disolvió en tetrahidrofurano (100 ml) y se añadió NaOH 2N (15.5 ml). La solución se agitó a temperatura ambiente durante 3.5 hr. Se añadieron 8 ml adicionales de NaOH 2N y la mezcla de reacción se calentó a 60°C durante 1.5 hr. La mezcla de reacción se enfrió y se concentró bajo vacío a un volumen pequeño y se dividió entre acetato de etilo y agua. La fase orgánica se lavó con agua (2x) y salmuera. Los extractos acuosos combinados se acidificaron con HCl 2N, se extrajeron en acetato de etilo (5x40 ml) y después se extrajo nuevamente con NaHCO₃ saturado (3x30 ml) y salmuera (30 ml). Las fases acuosas combinadas se acidificaron a pH 4.5 con HCl 2N y el sólido resultante se filtró y se lavó con agua para dar ácido 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahydro-1*H*-purin-1-il]pentanoico como un sólido color crema 2.19 g (38%).

La fase orgánica se secó sobre MgSO₄ y se concentró bajo vacío a una goma amarilla que se solidificó en reposo para dar 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahydro-1*H*-purin-1-il]pentanoato de metilo como un sólido color durazno (0.9 g, 15%)

Datos para ácido 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahydro-1*H*-purin-1-il]pentanoico:

LC/EM: m/z 377 [MH]⁺, TR 2.91 min

¹H RMN (DMSO-d₆) δ_H 0.90 (3H, t, J = 7Hz), 1.30 (2H, t, J = 7Hz), 1.45-1.68 (6H, m), 2.24 (2H, t, J = 7Hz), 3.89 (2H, t, J = 7Hz), 3.97 (2H, t, J = 7Hz), 12.00 (1 H, br s)

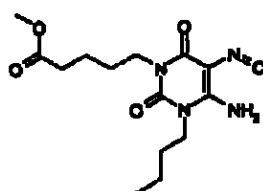
Datos para 5-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1*H*-purin-1-il]pentanoato de metilo:

LC/EM: m/z 391 $[MH]^+$, TR 3.15 min.

1H RMN ($CDCl_3$) δ_H 0.95 (3H, t, $J = 7Hz$), 1.27 (2H, t, $J = 7Hz$),
 5 1.38 (2H, m) 1.75 (2H, m), 2.06 (2H, s), 2.36 (2H, t, $J = 7Hz$), 3.62 (3H, s),
 4.03 (2H, br), 4.14 (2H, m)

c) 4-(4-Amino-3-butil-5-nitroso-2,6-dioxo-3,6-dihidro-1(2*H*)-pirimidinil)pentanoato de metilo

10



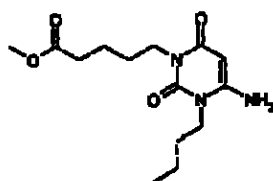
Preparado de modo similar a 4-(4-amino-3-butil-5-nitroso-2,6-dioxo-3,6-dihidro-1(2*H*)-pirimidinil)butanoato de etilo

15

LC/EM: m/z 327 $[MH]^+$, TR 2.54 min

d) 5-(4-Amino-3-butil-2,6-dioxo-3,6-dihidro-1(2*H*)-pirimidinil)pentanoato de metilo

20



Preparado de la misma manera que 4-(4-amino-3-butil-2,6-dioxo-3,6-dihidro-1(2*H*)-pirimidinil)butanoato de etilo

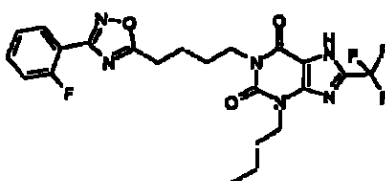
LC/EM: m/z 298 [MH]⁺, TR 2.47 min

EJEMPLO 9

3-Butil-1-(4-[3-(2-fluorofenil)-1,2,4-oxadiazol-5-il]butil)-8-(trifluorometil)-

5

3,7-dihidro-1H-purino-2,6-diona



Acido 5-[3-butyl-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-

10 purin-1-il]pentanoico (0.301 g, 0.8 mmoles) y CDI (0.153 g, 0.94 mmoles) se disolvieron en dimetilformamida anhidra (10 ml). Después de agitación de la solución bajo nitrógeno durante 4 hr a temperatura ambiente, se añadió 2-fluorobenzamidoxima (0.135 g, 0.88 mmoles) y la solución se calentó a 85°C durante 19 hr. La mezcla de reacción se concentró bajo vacío y se purificó sobre aminopropilo-SPE de 10 g (10 g) eluyendo con metanol. La evaporación del eluyente de metanol dio un aceite que se purificó adicionalmente por MDAP. Las fracciones pertinentes se combinaron y se concentraron bajo vacío y el residuo se secó por congelamiento a partir de dioxano para dar el compuesto del título (0.037 g, 9%) como un liofilato blanco.

20

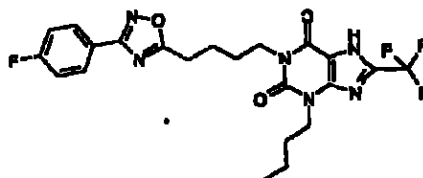
LC/EM: m/z 495 [MH]⁺, TR 3.68 min

¹H RMN (CDCl₃) δ_H 0.97 (3H, t, J = 7Hz), 1.40 (2H, m), 1.77 (2H, m), 1.88 (2H, m), 1.97 (2H, m), 3.05 (2H, t, J = 7Hz), 4.17 (2H, q, J = 7Hz), 7.19-7.29 (2H, m) 7.45-7.48 (1 H, m) 8.02, (1H, dt, J = 8 y 2Hz)

EJEMPL 10

3-Butil-1-[4-[3-(4-fluorofenil)-1,2,4-oxadiazol-5-il]butyl]-8-(trifluorometil)-
3,7-dihidro-1H-purino-2,6-diona

5



Acido 5-[3-butyl-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]pentanoico (0.301 g, 0.8 mmoles) y CDI (0.153 g, 0.94 mmoles) se disolvieron en dimetilformamida anhidra (10 ml). Después de agitar la solución
 10 bajo nitrógeno durante 4 hr a temperatura ambiente, se añadió 4-fluorobenzamidoxima (0.135 g, 0.88 mmoles) y la solución se calentó a 85°C durante 22 hr. La mezcla de reacción se concentró bajo vacío, se recogió en acetato de etilo y se lavó con salmuera (2x20 ml) y HCl 0.1 N. La fase orgánica se secó (MgSO₄) y se concentró bajo vacío para dar el producto
 15 requerido como un aceite amarillo pálido que se cristalizó en reposo. Este material se secó por congelamiento a partir de dioxano para dar el compuesto del título como un liofilato color crema (0.225 g, 57%).

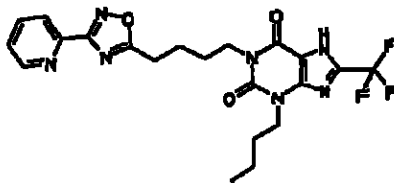
LC/EM: m/z 495 [MH]⁺, TR 3.77 min

¹H RMN (CDCl₃) δ_H 0.97 (3H, t, J = 7Hz), 1.27 (2H, m), 1.77 (2H, m), 1.87 (2H, m), 1.97 (2H, m), 3.02 (2H, t, J = 7Hz), 4.17 (4H, m), 7.15 (2H, m, J = 9Hz), 8.04 (2H, m).

20

EJEMPL 11**3-Butil-1-[4-[3-(2-piridinil)-1,2,4-oxadiazol-5-il]butil]-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona**

5



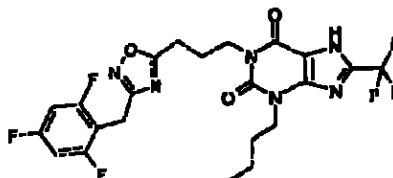
Acido 5-[3-butyl-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]pentanoico (0.15 g, 0.4 mmoles) y CDI (0.078 g, 0.48 mmoles) se
 10 disolvieron en dimetilformamida anhidra (5 ml). Después de agitar la solución bajo nitrógeno durante 1.5 hr a temperatura ambiente, se añadió 2-piridilamidoxima (0.06 g, 0.44 mmoles) y la solución se calentó a 90°C durante 16.5 hr. La mezcla de reacción se concentró bajo vacío y se purificó por MDAP. Las fracciones pertinentes se evaporaron para dar el compuesto del
 15 título como un sólido color canela (0.017 g, 9%).

LC/EM: m/z 478 [MH]⁺, TR 3.28 min

¹H RMN (CDCl₃) δ_H 0.95 (3H, t, J = 7Hz), 1.39 (2H, m, J = 7Hz),
 1.75 (2H, m), 1.84 (2H, m), 1.96 (2H, m), 3.07 (2H, t, J = 7Hz), 4.14 (4H, m),
 7.41 (1 H, m) 7.84 (1 H, dt, J = 8 y 2Hz), 8.10 (1 H, d, J = 8Hz), 8.75 (1 H, d, J
 20 = 4Hz)

EJEMPLO 12**3-Butil-8-(trifluorometil)-1-(3-{3-[(2,4,6-trifluorofenil)metil]-1,2,4-oxadiazol-5-il}propil)-3,7-dihidro-1H-purino-2,6-diona**

5



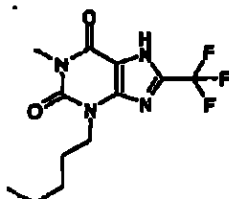
Acido 4-[3-butil-2,6-dioxo-8-(trifluorometil)-2,3,6,7-tetrahidro-1H-purin-1-il]butanoico (0.181 g, 0.5 mmoles) y CDI (0.089 g, 0.55 mmoles) se disolvieron en dimetilformamida anhidra (6 ml). Después de agitar la solución

10 bajo nitrógeno durante 3 hr a temperatura ambiente, se añadió 2,4,6-trifluorobencilamidoxima (0.112 g, 0.55 mmoles) y la solución se calentó a 90°C durante 20 hr, después a 110°C durante 6 hr. La mezcla de reacción se concentró bajo vacío a un aceite amarillo. Este se purificó en un Teledyne Isco CombiFlash® Companion® usando un cartucho de sílice RediSep de 35 g

15 (elución por gradientes cloroformo/etanol 0-15%). Las fracciones apropiadas se evaporaron bajo vacío para dar, después de secado por congelamiento a partir de dioxano, el compuesto del título como un sólido blanco (0.122 g, 46%).

LC/EM: m/z 531 [MH]⁺, TR 3.61 min

20 ¹H RMN (CDCl₃) δ_H 0.97 (3H, t, J = 7Hz), 1.41 (2H, m), 1.76 (2H, m), 2.23 (2H, m), 2.95 (2H, t, J = 8Hz), 4.00 (2H, s), 4.14 (2H, t, J = 8Hz), 4.22 (2H, t, J = 7Hz), 6.67 (2H, t, J = 7H z).

EJEMPLO 13**1-Metil-3-pentil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-diona**a) 1-Metil-3-pentil-8-(trifluorometil)-3,7-dihidro-1H-purino-2,6-5 diona

10 Una solución de 6-amino-3-metil-5-nitroso-1-pentil-2,4(1H,3H)-
 pirimidinodiona (0.042 g, 0.18 mmoles) en DCM anhidro (2 ml) se trató con
 anhídrido trifluoroacético (1 ml) y se dejó agitar a temperatura ambiente
 durante 10 minutos. La solución se concentró bajo vacío y el residuo se
 recogió en 1,4-dioxano (15 ml) y se hizo reaccionar con 10% de Pd/C (70 mg)
 15 y anhídrido trifluoroacético (0.1 ml) bajo una atmósfera de hidrógeno durante 6
 hr. La mezcla se filtró a través de celite y el filtrado se concentró, dando un
 aceite rojo. THF (2 ml) se añadió seguido por solución de NaOH 2M (ac). La
 mezcla se calentó a 50°C durante 10 minutos. Después de enfriamiento, la
 mezcla se dividió entre HCl 2M (ac) y EtOAc. La capa orgánica se separó, se
 20 lavó con salmuera, se secó (MgSO₄) y se concentró. El producto del título se
 purificó por MDAP para dar el compuesto del título como un sólido blanco (10
 mg, 19%).

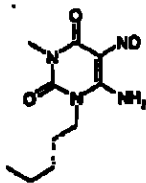
LC/EM m/z 305 [MH⁺], TR 3.2 mins.

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¹H RMN; (d⁶-DMSO) δ_H 0.92 (3H, t, J = 7 Hz), 1.38 (4H, m), 1.80 (2H, m), 3.51 (3H, s), 4.17 (2H, t, J = 8 Hz), 13.5 (1 H, br s).

b) 6-Amino-3-metil-5-nitroso-1-pentil-2,4(1*H*,3*H*)-pirimidinodiona

5



Se preparó de modo similar a 4-(4-amino-3-butil-5-nitroso-2,6-dioxo-3,6-dihidro-1(2*H*)-pirimidinil)butanoato de etilo.

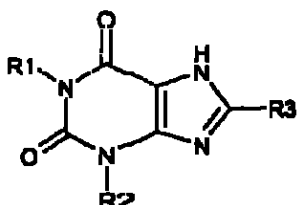
10

Todas las publicaciones, incluyendo pero sin limitarse a patentes y solicitudes de patente, citadas en esta especificación se incorporan aquí por referencia como si cada publicación individual fuera específicamente e individualmente indicada para ser incorporada aquí por referencia como si se expusiera por completo.

15

N UVIDAD DE LA INVENCION**REIVINDICACIONES**

5 1.- Por lo menos una entidad química seleccionada de compuestos de la fórmula (I)



10 (I)

y derivados farmacéuticamente aceptables de los mismos, en donde R¹ representa un grupo seleccionado de: hidrógeno, alquilo de C₁₋₁₀, alquenilo de C₂₋₁₀, alquinilo de C₂₋₁₀, y -(alq)_m-X-(alq)_n-Y, en donde X representa A, A1, A2 o un enlace directo; A representa un grupo seleccionado de: cicloalquileno, cicloalquenileno, arilo, heteroarilo, heterociclilo, -CH₂-OC(O)-; A1 representa un grupo seleccionado de: -CH₂-O-(CH₂)_q-arilo-O-, -CH₂-O-(CH₂)_w-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)-, -CH₂-(O)_p-(CH₂)_q-C(O)NR⁵-, -CH₂-N(R⁵)C(O)N(R⁵)-, -CH₂-C(O)N((CH₂)_wOH)-, -CH₂-NR⁵-S(O)₂-, CH₂-S(O)₂NR⁵-, -CH₂-C(O)O-, -O-, -NR⁵-, -S-; A2 representa: -CH(OH)-; cuando X es A, A1 o A2, Y representa un grupo seleccionado de: heteroarilo, heterociclilo, arilo, cicloalquilo, cicloalquenilo, -O(CH₂)_n-arilo, -C(O)O-arilo, -CH(arilo)₂, -CH(heteroarilo)₂, halogenoalquilo de C₁₋₆, -C(O)R⁴, -NR⁵R⁷, -C(O)NR⁵R⁷, -NR⁵C(O)R⁷, -NR⁵C(O)OR⁷, -C(O)(CH₂)_qOR⁴, halógeno, ciano, -N(R⁵)C(O)OR⁷,

15

20

$-\text{OC}(\text{O})\text{NR}^5\text{R}^6$, $-\text{NR}^5\text{C}(\text{O})\text{R}^6$, $-\text{OR}^5$, $-\text{OC}(\text{O})\text{R}^4$; cuando X es A1 y Y se selecciona de: $-\text{O}(\text{CH}_2)_n\text{-arilo}$, $-\text{O-heteroarilo}$, $-\text{OR}^5$, $-\text{OC}(\text{O})\text{R}^5$, $-\text{NH-arilo}$, $-\text{OC}(\text{O})\text{NR}^5\text{R}^6$, n es un entero seleccionado de 2, 3, 4 y 5; cuando X es A1 y Y es $-\text{CF}_3$, o cuando X es A2, n es un entero seleccionado de 1, 2, 3, 4 y 5;

5 cuando X es un enlace directo, Y representa un grupo seleccionado de: $-\text{C}(\text{O})(\text{CH}_2)_q\text{OR}^5$, $-\text{C}(\text{O})\text{-arilo}$, $-\text{C}(\text{O})\text{-heteroarilo}$, $-\text{C}(\text{O})\text{-heterociclilo}$, $-\text{heteroarilo}$, $-\text{heterociclilo}$, $-\text{arilo}$, $-\text{cicloalquilo}$, $-\text{cicloalquenilo}$, halogenoalquilo de C_{1-6} , $-\text{halógeno}$, $-\text{ciano}$, 3 ó 4 sistemas fusionados de anillo, $-\text{CH}(\text{arilo})_2$, $-\text{CH}(\text{heteroarilo})_2$, $-\text{OR}^5$, $-\text{NR}^5\text{R}^7$, $-\text{NCOOR}^8$, $-(\text{O})_p\text{C}(\text{O})\text{NR}^5\text{R}^6$, $-\text{MR}^5\text{C}(\text{O})\text{R}^6$, $-\text{OR}^5$, $-(\text{O})_p\text{C}(\text{O})\text{R}^4$; cuando Y incorpora un anillo, ese anillo puede ser

10 opcionalmente sustituido por uno o más de: alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , halógeno, $-\text{NH}_2$, $(\text{CH}_2)_q\text{-NR}^5\text{R}^7$, $-(\text{CH}_2)_q\text{-(O)}_p\text{-(CH}_2)_q\text{-N(R}^5\text{)C(O)OR}^8$, $-(\text{CH}_2)_q\text{-N(R}^5\text{)C(O)R}^8$, $-(\text{CH}_2)_q\text{-(O)}_p\text{-(CH}_2)_q\text{-C(O)NR}^5\text{R}^6$, $-(\text{CH}_2)_q\text{-N(R}^5\text{)C(O)N(R}^5\text{)R}^6$, $-(\text{CH}_2)_q\text{-C(O)N}((\text{CH}_2)_m\text{OH})\text{R}^5$, $-(\text{CH}_2)_q\text{-N(R}^5\text{)-S(O)}_2\text{R}^8$, $-\text{CH}_2\text{-S(O)}_2\text{N(R}^5\text{)R}^6$, $-\text{halogenoalquilo de } \text{C}_{1-6}$, $-\text{OCF}_3$, $-\text{OCH(F)}_2$, $-\text{OCH}_2\text{F}$, $-\text{COOR}^5$, $-\text{OR}^5$, $-(\text{R}^8)_p\text{CN}$, $-\text{S(O)}_2\text{R}^9$, $-(\text{CH}_2)_n\text{heteroarilo}$, $-(\text{CH}_2)_n\text{heterociclilo}$, $-(\text{CH}_2)_n\text{cicloalquilo}$, $-(\text{CH}_2)_n\text{cicloalquenilo}$, $-(\text{CH}_2)_n\text{arilo}$; R^2 se selecciona de:

15 hidrógeno; o alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, cicloalquenilo, heterociclilo, arilo, y heteroarilo, cada uno de los cuales puede

20 ser opcionalmente sustituido por uno o más de: alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, heterociclilo, arilo, heteroarilo, C_{1-6} halogenoalquilo, halógeno, $-\text{CN}$, $-\text{OR}^4$, $-(\text{CH}_2)_n\text{COR}^4$, $-\text{C}(\text{O})\text{OR}^4$, $-\text{OCOR}^4$, $-(\text{CH}_2)_n\text{NR}^5\text{R}^6$, $-(\text{NH})_p\text{CON R}^5\text{R}^6$, $-\text{OCONR}^5\text{R}^7$, y $-\text{NHC}(\text{O})\text{OR}^7$; R^3 se

selecciona de: alquilo de C₁₋₈ halogenado; R⁴ se selecciona de: hidrógeno, alquilo de C₁₋₈, alquenilo de C₂₋₈, alquinilo de C₂₋₈, -(CH₂)_n cicloalquilo, -(CH₂)_n cicloalquenilo, -(CH₂)_n heterociclilo, -(CH₂)_n arilo, y -(CH₂)_n heteroarilo; R⁵ y R⁶ se seleccionan de: hidrógeno y alquilo de C₁₋₄; R⁷ se selecciona de: hidrógeno, alquilo de C₁₋₈, alquenilo de C₂₋₈, alquinilo de C₂₋₈, -(CH₂)_t cicloalquilo, -(CH₂)_n cicloalquenilo, -(CH₂)_t heterociclilo, -(CH₂)_t arilo, y -(CH₂)_t heteroarilo; R⁸ se selecciona de alquilo de C₁₋₄; R⁹ se selecciona de alquilo de C₁₋₈, alquenilo de C₂₋₈, alquinilo de C₂₋₈, -(CH₂)_n cicloalquilo, -(CH₂)_n cicloalquenilo, -(CH₂)_n heterociclilo, -(CH₂)_n arilo, y -(CH₂)_n heteroarilo, CN; m representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5; n representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5; p representa un entero seleccionado de: 0 y 1; q representa un entero seleccionado de: 0, 1 y 2; t representa un entero seleccionado de: 1 y 2; w representa un entero seleccionado de: 2, 3 y 4; con la condición de que: i) cuando R¹ representa hidrógeno o alquilo de C₁₋₃, R² es diferente a R¹; ii) cuando R¹ representa etilo y R³ representa CF₃, R² es otro distinto de CH₂-CH=CH₂; y iii) cuando R¹ representa metilo y R³ representa CF₃, R² es otro distinto de i-butilo.

2.- Por lo menos una entidad química de conformidad con la reivindicación 1, caracterizada además porque R¹ se selecciona de: hidrógeno alquilo de C₁₋₁₀], y -(alq)_m-X-(alq)_n-Y.

3.- Por lo menos una entidad química de conformidad con la reivindicación 1 ó 2, caracterizada además porque X representa A o un enlace directo.

4.- Por lo menos una entidad química de conformidad con la reivindicación 1 ó 2, caracterizada además porque R^1 se selecciona de: hidrógeno y alquilo de C_{1-8} .

5 5.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones anteriores, caracterizada además porque R^2 se selecciona de: alquilo de C_{4-8} .

6.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones anteriores, caracterizada además porque R^3 se selecciona de: alquilo de C_{1-8} fluorado.

10 7.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones anteriores, caracterizada además porque R^3 representa CF_3 .

8.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones anteriores, para usarse en medicina
15 humana o veterinaria.

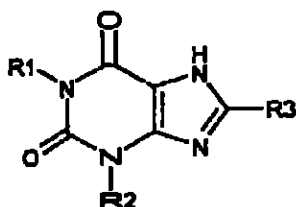
9.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-7, para usarse en el tratamiento de trastornos del metabolismo de lípidos incluyendo dislipidemia e hiperlipoproteínaemia y/o de enfermedades o condiciones inflamatorias.

20 10.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-7, para usarse en el tratamiento de dislipidemia diabética, dislipidemia mixta, insuficiencia cardíaca, hipercolesterolemia, enfermedad cardiovascular incluyendo aterosclerosis,

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arteriosclerosis, e hipertrigliceridemia, diabetes mellitus de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad, enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica o accidente cerebrovascular.

- 5 11.- Por lo menos una entidad química seleccionada de compuestos de la fórmula (I)



10

(I)

- y derivados farmacéuticamente aceptables de los mismos, en donde R¹ representa un grupo seleccionado de: hidrógeno, alquilo de C₁₋₁₀, alquenilo de C₂₋₁₀, alquinilo de C₂₋₁₀, y -(alq)_m-X-(alq)_n-Y, en donde X representa A, A1, A2 o un enlace directo; A representa un grupo seleccionado de: cicloalquileno, cicloalquenileno, arilo, heteroarilo, heterociclilo, -CH₂-OC(O)-; A1 representa un grupo seleccionado de: -CH₂-O-(CH₂)_qarilo-O-, -CH₂-O-(CH₂)_wN(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)-, -CH₂-(O)_p-(CH₂)_qC(O)NR⁵-, -CH₂-N(R⁵)C(O)N(R⁵)-, -CH₂-C(O)N((CH₂)_wOH)-, -CH₂-NR⁵-S(O)₂-, CH₂-S(O)₂NR⁵-, -CH₂-C(O)O-, -O-, -NR⁵-, -S-; A2 representa: -CH(OH)-; cuando X es A, A1 o A2, Y representa un grupo seleccionado de: heteroarilo, heterociclilo, arilo, cicloalquilo, cicloalquenilo, -O(CH₂)_n-arilo, -C(O)O-arilo, -CH(arilo)₂, -CH(heteroarilo)₂, halogenoalquilo de C₁₋₆, -C(O)R⁴, -NR⁵R⁷, -C(O)NR⁵R⁷, -NR⁵C(O)R⁷, -NR⁵C(O)OR⁷, -C(O)(CH₂)_qOR⁴, halógeno, ciano, -N(R⁵)C(O)OR⁷,
- 15
- 20

$-\text{OC}(\text{O})\text{NR}^5\text{R}^6$, $-\text{NR}^5\text{C}(\text{O})\text{R}^6$, $-\text{OR}^5$, $-\text{OC}(\text{O})\text{R}^4$; cuando X es A1 y Y se selecciona de: $-\text{O}(\text{CH}_2)_n\text{-arilo}$, $-\text{O-heteroarilo}$, $-\text{OR}^5$, $-\text{OC}(\text{O})\text{R}^5$, $-\text{NH-arilo}$, $-\text{OC}(\text{O})\text{NR}^5\text{R}^6$, n es un entero seleccionado de 2, 3, 4 y 5; cuando X es A1 y Y es $-\text{CF}_3$, o cuando X es A2, n es un entero seleccionado de 1, 2, 3, 4 y 5;

5 cuando X es un enlace directo, Y representa un grupo seleccionado de: $-\text{C}(\text{O})(\text{CH}_2)_q\text{OR}^5$, $-\text{C}(\text{O})\text{-arilo}$, $-\text{C}(\text{O})\text{-heteroarilo}$, $-\text{C}(\text{O})\text{-heterociclilo}$, $-\text{heteroarilo}$, $-\text{heterociclilo}$, $-\text{arilo}$, $-\text{cicloalquilo}$, $-\text{cicloalquenilo}$, halogenoalquilo de C_{1-8} , $-\text{halógeno}$, $-\text{ciano}$, 3 ó 4 sistemas fusionados de anillo, $-\text{CH}(\text{arilo})_2$, $-\text{CH}(\text{heteroarilo})_2$, $-\text{OR}^5$, $-\text{NR}^5\text{R}^7$, $-\text{NCOOR}^8$, $-(\text{O})_p\text{C}(\text{O})\text{NR}^5\text{R}^6$, $-\text{MR}^5\text{C}(\text{O})\text{R}^8$, $-\text{OR}^5$, $-(\text{O})_p\text{C}(\text{O})\text{R}^4$; cuando Y incorpora un anillo, ese anillo puede ser

10 opcionalmente sustituido por uno o más de: alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , halógeno, $-\text{NH}_2$, $(\text{CH}_2)_q\text{-NR}^5\text{R}^7$, $-(\text{CH}_2)_q\text{-(O)}_p\text{-(CH}_2)_q\text{-N(R}^5\text{)C(O)OR}^8$, $-(\text{CH}_2)_q\text{-N(R}^5\text{)C(O)R}^8$, $-(\text{CH}_2)_q\text{-(O)}_p\text{-(CH}_2)_q\text{-C(O)NR}^5\text{R}^6$, $-(\text{CH}_2)_q\text{-N(R}^5\text{)C(O)N(R}^5\text{)R}^8$, $-(\text{CH}_2)_q\text{-C(O)N}((\text{CH}_2)_m\text{OH})\text{R}^5$, $-(\text{CH}_2)_q\text{-N(R}^5\text{)-S(O)}_2\text{R}^8$, $-\text{CH}_2\text{-S(O)}_2\text{N(R}^5\text{)R}^8$, $-\text{halogenoalquilo de } \text{C}_{1-8}$, $-\text{OCF}_3$, $-\text{OCH(F)}_2$, $-\text{OCH}_2\text{F}$, $-\text{COOR}^5$, $-\text{OR}^5$, $-(\text{R}^8)_p\text{CN}$, $-\text{S(O)}_2\text{R}^8$, $-(\text{CH}_2)_n\text{heteroarilo}$, $-(\text{CH}_2)_n\text{heterociclilo}$, $-(\text{CH}_2)_n\text{cicloalquilo}$, $-(\text{CH}_2)_n\text{cicloalquenilo}$, $-(\text{CH}_2)_n\text{arilo}$; R^2 se selecciona de:

15 hidrógeno; o alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, cicloalquenilo, heterociclilo, arilo, y heteroarilo, cada uno de los cuales puede

20 ser opcionalmente sustituido por uno o más de: alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, heterociclilo, arilo, heteroarilo, C_{1-8} halogenoalquilo, halógeno, $-\text{CN}$, $-\text{OR}^4$, $-(\text{CH}_2)_n\text{COR}^4$, $-\text{C}(\text{O})\text{OR}^4$, $-\text{OCOR}^4$, $-(\text{CH}_2)_n\text{NR}^5\text{R}^6$, $-(\text{NH})_p\text{CON R}^5\text{R}^8$, $-\text{OCONR}^5\text{R}^7$, y $-\text{NHC}(\text{O})\text{OR}^7$; R^3 se

selecciona de: alquilo de C_{1-6} halogenado; R^4 se selecciona de: hidrógeno, alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_n$ heterociclilo, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo; R^5 y R^6 se seleccionan de: hidrógeno y alquilo de C_{1-4} ; R^7 se selecciona de:

5 hidrógeno, alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_t$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_t$ heterociclilo, $-(CH_2)_t$ arilo, y $-(CH_2)_t$ heteroarilo; R^8 se selecciona de alquilo de C_{1-4} ; R^9 se selecciona de alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_n$ heterociclilo, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo,

10 CN; m representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5; n representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5; p representa un entero seleccionado de: 0 y 1; q representa un entero seleccionado de: 0, 1 y 2; t representa un entero seleccionado de: 1 y 2; w representa un entero seleccionado de: 2, 3 y 4; para usarse en la fabricación de un medicamento para tratar dislipidemia

15 diabética, dislipidemia mixta, insuficiencia cardiaca, hipercolesteraemia, enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis e hipertrigliceridemia, diabetes mellitus de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad, enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica o accidente

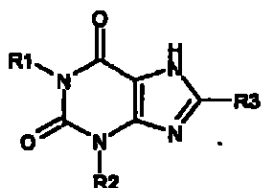
20 cerebrovascular.

12.- Un método para el tratamiento de un sujeto humano o animal que tiene una condición en donde la sub-activación del receptor de HM74A contribuye a la condición o en donde la activación del receptor será

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benéfica, dicho método comprende administrar a dicho sujeto humano o animal una cantidad efectiva de por lo menos una entidad química seleccionada de: compuestos de la fórmula (II)

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(II)

y derivados farmacéuticamente aceptables de los mismos, en donde R¹ representa un grupo seleccionado de: hidrógeno, alquilo de C₁₋₁₀, alquenilo de C₂₋₁₀, alquinilo de C₂₋₁₀, y -(alq)_m-X-(alq)_n-Y, en donde X representa A, A1, A2 o un enlace directo; A representa un grupo seleccionado de: cicloalquileno, cicloalquenileno, arilo, heteroarilo, heterociclilo, -CH₂-OC(O)-; A1 representa un grupo seleccionado de: -CH₂-O-(CH₂)_qarilo-O-, -CH₂-O-(CH₂)_wN(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)-, -CH₂-(O)_p-(CH₂)_qC(O)NR⁵-, -CH₂-N(R⁵)C(O)N(R⁵)-, -CH₂-C(O)N((CH₂)_wOH)-, -CH₂-NR⁵-S(O)₂-, CH₂-S(O)₂NR⁵-, -CH₂-C(O)O-, -O-, -NR⁵-, -S-; A2 representa: -CH(OH)-; cuando X es A, A1 o A2, Y representa un grupo seleccionado de: heteroarilo, heterociclilo, arilo, cicloalquilo, cicloalquenilo, -O(CH₂)_n-arilo, -C(O)O-arilo, -CH(arilo)₂, -CH(heteroarilo)₂, halogenoalquilo de C₁₋₆, -C(O)R⁴, -NR⁵R⁷, -C(O)NR⁵R⁷, -NR⁵C(O)R⁷, -NR⁵C(O)OR⁷, -C(O)(CH₂)_qOR⁴, halógeno, ciano, -N(R⁵)C(O)OR⁷, -OC(O)NR⁵R⁶, -NR⁵C(O)R⁶, -OR⁵, -OC(O)R⁴; cuando X es A1 y Y se selecciona de: -O(CH₂)_n-arilo, -O-heteroarilo, -OR⁵, -OC(O)R⁵, -NH-arilo, -OC(O)NR⁵R⁶, n es un entero seleccionado de 2, 3, 4 y 5; cuando X es A1 y Y

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es $-\text{CF}_3$, o cuando X es A2, n es un entero seleccionado de 1, 2, 3, 4 y 5; cuando X es un enlace directo, Y representa un grupo seleccionado de: $-\text{C}(\text{O})(\text{CH}_2)_q\text{OR}^5$, $-\text{C}(\text{O})$ -arilo, $-\text{C}(\text{O})$ -heteroarilo, $-\text{C}(\text{O})$ -heterociclilo, -heteroarilo, -heterociclilo, -arilo, -cicloalquilo, -cicloalquenilo, halogenoalquilo de C_{1-8} , -halógeno, -ciano, 3 ó 4 sistemas fusionados de anillo, $-\text{CH}(\text{arilo})_2$, $-\text{CH}(\text{heteroarilo})_2$, $-\text{OR}^5$, $-\text{NR}^5\text{R}^7$, $-\text{NCOOR}^8$, $-(\text{O})_p\text{C}(\text{O})\text{NR}^5\text{R}^8$, $-\text{MR}^5\text{C}(\text{O})\text{R}^8$, $-\text{OR}^5$, $-(\text{O})_p\text{C}(\text{O})\text{R}^4$; cuando Y incorpora un anillo, ese anillo puede ser opcionalmente sustituido por uno o más de: alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , halógeno, $-\text{NH}_2$, $(\text{CH}_2)_q\text{NR}^5\text{R}^7$, $-(\text{CH}_2)_q(\text{O})_p(\text{CH}_2)_q\text{N}(\text{R}^5)\text{C}(\text{O})\text{OR}^8$, $-(\text{CH}_2)_q\text{N}(\text{R}^5)\text{C}(\text{O})\text{R}^8$, $-(\text{CH}_2)_q(\text{O})_p(\text{CH}_2)_q\text{C}(\text{O})\text{NR}^5\text{R}^8$, $-(\text{CH}_2)_q\text{N}(\text{R}^5)\text{C}(\text{O})\text{N}(\text{R}^5)\text{R}^6$, $-(\text{CH}_2)_q\text{C}(\text{O})\text{N}((\text{CH}_2)_m\text{OH})\text{R}^5$, $-(\text{CH}_2)_q\text{N}(\text{R}^5)\text{S}(\text{O})_2\text{R}^8$, $-\text{CH}_2\text{S}(\text{O})_2\text{N}(\text{R}^5)\text{R}^6$, - halogenoalquilo de C_{1-8} , $-\text{OCF}_3$, $-\text{OCH}(\text{F})_2$, $-\text{OCH}_2\text{F}$, $-\text{COOR}^5$, $-\text{OR}^5$, $-(\text{R}^5)_p\text{CN}$, $-\text{S}(\text{O})_2\text{R}^9$, $-(\text{CH}_2)_n\text{heteroarilo}$, $-(\text{CH}_2)_n\text{heterociclilo}$, $-(\text{CH}_2)_n\text{cicloalquilo}$, $-(\text{CH}_2)_n\text{cicloalquenilo}$, $-(\text{CH}_2)_n\text{arilo}$; R^2 se selecciona de: hidrógeno; o alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, cicloalquenilo, heterociclilo, arilo, y heteroarilo, cada uno de los cuales puede ser opcionalmente sustituido por uno o más de: alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, heterociclilo, arilo, heteroarilo, C_{1-8} halogenoalquilo, halógeno, $-\text{CN}$, $-\text{OR}^4$, $-(\text{CH}_2)_n\text{COR}^4$, $-\text{C}(\text{O})\text{OR}^4$, $-\text{OCOR}^4$, $-(\text{CH}_2)_n\text{NR}^5\text{R}^8$, $-(\text{NH})_p\text{CON R}^5\text{R}^8$, $-\text{OCONR}^5\text{R}^7$, y $-\text{NHC}(\text{O})\text{OR}^7$; R^3 se selecciona de: alquilo de C_{1-8} halogenado; R^4 se selecciona de: hidrógeno, alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , $-(\text{CH}_2)_n$ cicloalquilo, $-(\text{CH}_2)_n$ cicloalquenilo, $-(\text{CH}_2)_n$ heterociclilo, $-(\text{CH}_2)_n$ arilo, y $-(\text{CH}_2)_n$ heteroarilo; R^5 y R^8

se seleccionan de: hidrógeno y alquilo de C_{1-4} ; R^7 se selecciona de: hidrógeno, alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , $-(CH_2)_t$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_t$ heterocíclico, $-(CH_2)_t$ arilo, y $-(CH_2)_t$ heteroarilo; R^8 se selecciona de alquilo de C_{1-4} ; R^9 se selecciona de alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_n$ heterocíclico, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo, CN; m representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5; n representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5; p representa un entero seleccionado de: 0 y 1; q representa un entero seleccionado de: 0, 1 y 2; t representa un entero seleccionado de: 1 y 2; w representa un entero seleccionado de: 2, 3 y 4.

13.- El método de conformidad con la reivindicación 12, en donde el sujeto humano o animal tiene un trastorno del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteínaemia o una enfermedad o condición inflamatoria.

14.- Una formulación farmacéutica que comprende por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-7 y uno o más diluyentes, excipientes o vehículos farmacéuticamente aceptables.

15.- Una combinación para administrarse junta o por separado, secuencialmente o simultáneamente, en formulaciones farmacéuticas separadas o combinadas, dicha combinación comprende por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-7

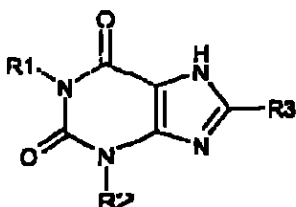
junto con otro agente terapéuticamente activo.

16.- Una formulación farmacéutica que comprende: (i) por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-7; (ii) uno o más ingredientes activos seleccionados de
5 estatinas, fibratos, resinas de unión de ácido biliar y ácido nicotínico; y (iii) uno o más diluyentes, excipientes o vehículos farmacéuticamente aceptables.

Reivndicaciones enmendadas

1.- Por lo menos una entidad química seleccionada de compuestos de la fórmula (I)

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(I)

y derivados farmacéuticamente aceptables de los mismos, en donde R¹
 10 representa un grupo seleccionado de: hidrógeno, alquilo de C₁₋₁₀, alquenilo de C₂₋₁₀, alquinilo de C₂₋₁₀, y -(alq)_m-X-(alq)_n-Y, en donde X representa A, A1, A2 o un enlace directo; A representa un grupo seleccionado de: cicloalquileo, cicloalquenileo, arilo, heteroarilo, heterociclilo, -CH₂-OC(O)-; A1 representa un grupo seleccionado de: -CH₂-O-(CH₂)_qarilo-O-, -CH₂-O-(CH₂)_wN(R⁵)C(O)O-,
 15 -CH₂-N(R⁵)C(O)O-, -CH₂-N(R⁵)C(O)-, -CH₂-(O)_p-(CH₂)_qC(O)NR⁵-, -CH₂-N(R⁵)C(O)N(R⁵)-, -CH₂-C(O)N((CH₂)_wOH)-, -CH₂-NR⁵-S(O)₂-, CH₂-S(O)₂NR⁵-, -CH₂-C(O)O-, -O-, -NR⁵-, -S-; A2 representa: -CH(OH)-; cuando X es A, A1 o A2, Y representa un grupo seleccionado de: heteroarilo, heterociclilo, arilo, cicloalquilo, cicloalquenilo, -O(CH₂)_n-arilo, -C(O)O-arilo, -CH(arilo)₂, -
 20 CH(heteroarilo)₂, halogenoalquilo de C₁₋₆, -C(O)R⁴, -NR⁵R⁷, -C(O)NR⁵R⁷, -NR⁵C(O)R⁷, -NR⁵C(O)OR⁷, -C(O)(CH₂)_qOR⁴, halógeno, ciano, -N(R⁵)C(O)OR⁷, -OC(O)NR⁵R⁶, -NR⁵C(O)R⁶, -OR⁵, -OC(O)R⁴; cuando X es A1 y Y se selecciona de: -O(CH₂)_n-arilo, -O-heteroarilo, -OR⁵, -OC(O)R⁵, -NH-arilo, -

$OC(O)NR^5R^6$, n es un entero seleccionado de 2, 3, 4 y 5; cuando X es A1 y Y es $-CF_3$, o cuando X es A2, n es un entero seleccionado de 1, 2, 3, 4 y 5; cuando X es un enlace directo, Y representa un grupo seleccionado de: $-C(O)(CH_2)_qOR^5$, $-C(O)$ -arilo, $-C(O)$ -heteroarilo, $-C(O)$ -heterociclilo, -heteroarilo, -heterociclilo, -arilo, -cicloalquilo, -cicloalquenilo, halogenoalquilo de C_{1-8} , -halógeno, -ciano, 3 ó 4 sistemas fusionados de anillo, $-CH(arilo)_2$, $-CH(heteroarilo)_2$, $-OR^5$, $-NR^5R^7$, $-NCOOR^8$, $-(O)_pC(O)NR^5R^6$, $-MR^5C(O)R^8$, $-OR^5$, $-(O)_pC(O)R^4$; cuando Y incorpora un anillo, ese anillo puede ser opcionalmente sustituido por uno o más de: alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , halógeno, $-NH_2$, $(CH_2)_qNR^5R^7$, $-(CH_2)_q-(O)_p-(CH_2)_qN(R^5)C(O)OR^8$, $-(CH_2)_q-N(R^5)C(O)R^8$, $-(CH_2)_q-(O)_p-(CH_2)_q-C(O)NR^5R^6$, $-(CH_2)_q-N(R^5)C(O)N(R^5)R^6$, $-(CH_2)_q-C(O)N((CH_2)_mOH)R^5$, $-(CH_2)_q-N(R^5)-S(O)_2R^8$, $-CH_2-S(O)_2N(R^5)R^6$, - halogenoalquilo de C_{1-8} , $-OCF_3$, $-OCH(F)_2$, $-OCH_2F$, $-COOR^5$, $-OR^5$, $-(R^8)_pCN$, $-S(O)_2R^9$, $-(CH_2)_n$ heteroarilo, $-(CH_2)_n$ heterociclilo $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_n$ arilo; R^2 se selecciona de: hidrógeno; o alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, cicloalquenilo, heterociclilo, arilo, y heteroarilo, cada uno de los cuales puede ser opcionalmente sustituido por uno o más de: alquilo de C_{1-10} , alquenilo de C_{2-10} , alquinilo de C_{2-10} , cicloalquilo, heterociclilo, arilo, heteroarilo, C_{1-8} halogenoalquilo, halógeno, $-CN$, $-OR^4$, $-(CH_2)_nCOR^4$, $-C(O)OR^4$, $-OCOR^4$, $-(CH_2)_nNR^5R^6$, $-(NH)_pCONR^5R^6$, $-OCONR^5R^7$, y $-NHC(O)OR^7$; R^3 se selecciona de: alquilo de C_{1-8} halogenado; R^4 se selecciona de: hidrógeno, alquilo de C_{1-8} , alquenilo de C_{2-8} , alquinilo de C_{2-8} , $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$

cicloalquenilo, $-(CH_2)_n$ heterociclilo, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo; R^5 y R^6 se seleccionan de: hidrógeno y alquilo de C_{1-4} ; R^7 se selecciona de: hidrógeno, alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_t$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_t$ heterociclilo, $-(CH_2)_t$ arilo, y $-(CH_2)_t$ heteroarilo; R^8 se selecciona de alquilo de C_{1-4} ; R^9 se selecciona de alquilo de C_{1-6} , alquenilo de C_{2-6} , alquinilo de C_{2-6} , $-(CH_2)_n$ cicloalquilo, $-(CH_2)_n$ cicloalquenilo, $-(CH_2)_n$ heterociclilo, $-(CH_2)_n$ arilo, y $-(CH_2)_n$ heteroarilo, CN; m representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5; n representa un entero seleccionado de: 0, 1, 2, 3, 4 y 5; p representa un entero seleccionado de: 0 y 1; q representa un entero seleccionado de: 0, 1 y 2; t representa un entero seleccionado de: 1 y 2; w representa un entero seleccionado de: 2, 3 y 4; con la condición de que: i) cuando R^1 representa hidrógeno o alquilo de C_{1-3} , R^2 es diferente a R^1 ; ii) cuando R^1 representa etilo y R^3 representa CF_3 , R^2 es otro distinto de $CH_2-CH=CH_2$; y iii) cuando R^1 representa metilo y R^3 representa CF_3 , R^2 es otro distinto de i-butilo.

2.- Por lo menos una entidad química de conformidad con la reivindicación 1, caracterizada además porque R^1 se selecciona de: hidrógeno alquilo de C_{1-10} , y $-(alq)_m-X-(alq)_n-Y$.

3.- Por lo menos una entidad química de conformidad con la reivindicación 1 ó 2, caracterizada además porque X representa A o un enlace directo.

4.- Por lo menos una entidad química de conformidad con la reivindicación 1 ó 2, caracterizada además porque R^1 se selecciona de:

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hidrógeno y alquilo de C₁₋₈.

5.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones anteriores, caracterizada además porque R² se selecciona de: alquilo de C₄₋₈.

5 6.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones anteriores, caracterizada además porque R³ representa CF₃.

7.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones anteriores, para usarse en medicina
10 humana o veterinaria.

8.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-6, para usarse en el tratamiento de trastornos del metabolismo de lípidos incluyendo dislipidemia e hiperlipoproteínaemia y/o de enfermedades o condiciones inflamatorias.

15 9.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-6, para usarse en el tratamiento de dislipidemia diabética, dislipidemia mixta, insuficiencia cardíaca, hipercolesterolemia, enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis, e hipertrigliceridemia, diabetes mellitus de tipo II, diabetes de
20 tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad, enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica o accidente cerebrovascular.

10.- Por lo menos una entidad química de conformidad con

cualquiera de las reivindicaciones 1-6, para usarse en la fabricación de un medicamento para el tratamiento de trastornos del metabolismo de lípidos incluyendo dislipidemia e hiperlipoproteínaemia y/o de enfermedades o condiciones inflamatorias.

5 11.- Por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-6, para usarse en la fabricación de un medicamento para el tratamiento de dislipidemia diabética, dislipidemia mixta, insuficiencia cardíaca, hipercolesterolemia, enfermedad cardiovascular incluyendo aterosclerosis, arteriosclerosis, e hipertrigliceridemia, diabetes
10 mellitus de tipo II, diabetes de tipo I, resistencia a la insulina, hiperlipidemia, anorexia nerviosa, obesidad, enfermedad de arteria coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular periférica o accidente cerebrovascular.

 12.- Por lo menos una entidad química de conformidad con
15 cualquiera de las reivindicaciones 1-6, para usarse en la fabricación de un medicamento para tratar una condición en donde la sub-activación del receptor de HM74A contribuye a la condición o en donde la activación del receptor será benéfica.

 13.- El compuesto de la fórmula (II) de conformidad con la
20 reivindicación 10, para usarse en el tratamiento de una condición en donde la sub-activación del receptor de HM74A contribuye a la condición o en donde la activación del receptor será benéfica.

 14.- Una formulación farmacéutica que comprende por lo menos

una entidad química de conformidad con cualquiera de las reivindicaciones 1-6 y uno o más diluyentes, excipientes o vehículos farmacéuticamente aceptables.

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15 15.- Una combinación para administrarse junta o por separado, secuencialmente o simultáneamente, en formulaciones farmacéuticas separadas o combinadas, dicha combinación comprende por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-6 junto con otro agente terapéuticamente activo.

10 16.- Una formulación farmacéutica que comprende: (i) por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-6; (ii) uno o más ingredientes activos seleccionados de estatinas, fibratos, resinas de unión de ácido biliar y ácido nicotínico; y (iii) uno o más diluyentes, excipientes o vehículos farmacéuticamente aceptables.

15 17.- Un método para el tratamiento de un sujeto humano o animal que tiene una condición en donde la sub-activación del receptor de HM74A contribuye a la condición o en donde la activación del receptor será benéfica, dicho método comprende administrar a dicho sujeto humano o animal una cantidad efectiva de por lo menos una entidad química de conformidad con cualquiera de las reivindicaciones 1-6.

20 18.- El método de conformidad con la reivindicación 17, en donde el sujeto humano o animal tiene un trastorno del metabolismo de lípidos incluyendo dislipidemia o hiperlipoproteínaemia o una enfermedad o condición inflamatoria.

19.- El método de conformidad con la reivindicación 17, en
donde el sujeto humano o animal tiene un trastorno de dislipidemia diabética,
dislipidemia mixta, insuficiencia cardíaca, hipercolesterolemia, enfermedad
cardiovascular incluyendo aterosclerosis, arteriosclerosis, e
5 hipertrigliceridemia, diabetes mellitus de tipo II, diabetes de tipo I, resistencia a
la insulina, hiperlipidemia, anorexia nerviosa, obesidad, enfermedad de arteria
coronaria, trombosis, angina, insuficiencia renal crónica, enfermedad vascular
periférica o accidente cerebrovascular.

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RESUMEN DE LA INVENCION

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La presente invención se refiere a compuestos terapéuticamente activos de la fórmula (I) que son derivados de xantina, procedimientos para la
5 fabricación de dichos derivados, formulaciones farmacéuticas que contienen los compuestos activos y el uso de los compuestos en terapia, particularmente en el tratamiento de enfermedades en donde la sub-activación del receptor de HM74A contribuye a la enfermedad o en donde la activación del receptor será benéfica.

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P07/713F

PATENT COOPERATION TREATY

PCT

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference LXH/PB61093	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, Item 5 below.	
International application No. PCT/EP2005/011376	International filing date (day/month/year) 20/10/2005	(Earliest) Priority Date (day/month/year) 22/10/2004
Applicant SMITHKLINE BEECHAM CORPORATION		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☒ Certain claims were found unsearchable (See Box II).

3. ☐ Unity of invention is lacking (see Box III).

4. With regard to the title,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

XANTHINE DERIVATIVES WITH HM74A RECEPTOR ACTIVITY

5. With regard to the abstract,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

a. the figure of the drawings to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

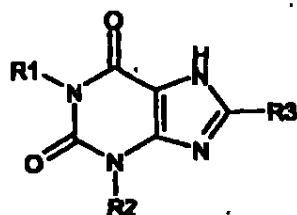
☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The present invention relates to therapeutically active compounds of formula (I) which are xanthine derivatives, processes for the manufacture of said derivatives, pharmaceutical formulations containing the active compounds and the use of the compounds in therapy, particularly in the treatment of diseases where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial.



(I)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/011376

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A. CLASSIFICATION OF SUBJECT MATTER C07D473/04 C07D473/06 A61K31/522 A61P9/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C07D A61K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KRAMER G L ET AL: "Selective inhibition of cyclic nucleotide phosphodiesterases by analogues of 1-methyl-3-isobutylxanthine" BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 16, no. 15, 1977, pages 3316-3321, XP002169138 ISSN: 0006-2960 Compound 12 ----- -/-	1,2,4, 6-14
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (see specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "Z" document member of the same patent family		
Date of the actual completion of the international search 26 January 2006		Date of mailing of the international search report 22/02/2006
Name and mailing address of the ISA/ European Patent Office, P.O. 5818 Patentamt 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer De Jong, B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/011376

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

Although claims 12,13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2005/011376

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0103497	A	21-03-1984	
		AU 560809 B2	16-04-1987
		AU 1736183 A	02-02-1984
		CA 1207766 A1	15-07-1986
		DD 216934 A5	02-01-1985
		DE 3362022 D1	13-03-1986
		DK 342883 A	29-01-1984
		ES 8500275 A1	01-01-1985
		FR 2531085 A1	03-02-1984
		GR 78891 A1	02-10-1984
		HU 188353 B	28-04-1986
		IE 55773 B1	16-01-1991
		IL 69362 A	31-07-1986
		JP 1692068 C	27-08-1992
		JP 3059903 B	12-09-1991
		JP 59042383 A	08-03-1984
		KR 8701268 B1	30-06-1987
		MA 19860 A1	01-04-1984
		NO 832742 A	30-01-1984
		NZ 205035 A	11-04-1986
		OA 7508 A	31-03-1985
		PH 19275 A	21-02-1986
		PT 77110 A	01-08-1983
		SU 1240361 A3	23-06-1986
		SU 1245261 A3	15-07-1986
		US 4548820 A	22-10-1985
		ZA 8305496 A	28-03-1984
US 2879271	A	24-03-1959	NONE
WO 02084298	A	24-10-2002	
		EP 1377834 A2	07-01-2004
		US 2004254224 A1	16-12-2004

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/EP2005/011376

International filing date (day/month/year)
20.10.2005

Priority date (day/month/year)
22.10.2004

International Patent Classification (IPC) or both national classification and IPC
C07D473D04, C07D473D06, A61K31/522, A61P9/00

Applicant
SMITHKLINE BEECHAM CORPORATION

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 56.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer De Jong, B Telephone No. +31 70 340-2833 
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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No. **162**
PCT/EP2005/011376

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ in written format
 - ☐ in computer readable form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 12,13

because:

☒ the said international application, or the said claims Nos. 12,13 (with respect to industrial application) relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form

☐ has not been furnished

☐ does not comply with the standard

the computer readable form

☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

☒ See separate sheet for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/011376

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**Box No. V Reasoned statement under Rule 43b/s.1(a)(i) with regard to novelty, inventive step or
Industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	3,15,16
	No: Claims	1,2,4-14
Inventive step (IS)	Yes: Claims	3,15,16
	No: Claims	1,2,4-14
Industrial applicability (IA)	Yes: Claims	1-11,14-16
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item III.

Claims 12,13 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V.

Reference is made to the following documents:

- D1: KRAMER G L ET AL: "Selective inhibition of cyclic nucleotide phosphodiesterases by analogues of 1-methyl-3-isobutylxanthine" BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 16, no. 15, 1977, pages 3316-3321, XP002169138 ISSN: 0006-2960
- D2: WELLS, JACK N. ET AL: "Inhibition of separated forms of cyclic nucleotide phosphodiesterase from pig coronary arteries by 1,3-disubstituted and 1,3,8-trisubstituted xanthines" JOURNAL OF MEDICINAL CHEMISTRY, 24(8), 954-8 CODEN: JMCMAJ; ISSN: 0022-2623, 1981, XP002169139
- D3: JACOBSON K A ET AL: "Effect of trifluoromethyl and other substituents on activity of xanthines at adenosine receptors" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 36, 1993, pages 2639-2644, XP002327691 ISSN: 0022-2623
- D4: CORSANO, STEFANO ET AL: "Structure-activity relationships in a series of 8-substituted xanthines as bronchodilator and A1-adenosine receptor antagonists" ARCHIV DER PHARMAZIE (WEINHEIM, GERMANY), 328(9), 654-8 CODEN: ARPMAS; ISSN: 0365-6233, 1995, XP008058976
- D5: REGNIER G L ET AL: "New xanthine derivatives with potent and long lasting anti-bronchoconstrictive activity" EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 22, 1987, pages 243-250, XP002291236 ISSN: 0223-5234
- D6: EP-A-0 103 497 (ADIR) 21 March 1984 (1984-03-21)
- D7: BREDERECK, HELLMUT ET AL: "Syntheses in the purine series. XIII. The preparation of several xanthine-8-aldehydes" CHEMISCHE BERICHTE, 95, 403-13 CODEN: CHBEAM; ISSN: 0009-2940, 1962, XP002364488
- D8: US-A-2 879 271 (KALLISCHNIGG ROLF) 24 March 1959 (1959-03-24)

D9: WO 02/084298 A (GLAXO GROUP LIMITED; FOORD, STEVEN, MICHAEL;
PIKE, NICHOLAS, BRIAN; WI) 24 October 2002 (2002-10-24)

Novelty

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1,2,4,6-14 is not new in the sense of Article 33(2) PCT:

D1 discloses compound 12, which is an inhibitor of phosphodiesterase. It is commonly known that PDE inhibitors can be used against cardiovascular diseases such as e.g. dyslipidemia and atherosclerosis. This teaching is therefore novelty destroying for claims 1,2,4,6-14.

D2 discloses compound 4, which is an inhibitor of phosphodiesterase. This teaching is novelty destroying for claims 1,2,4,5,8-14.

D3 discloses the compounds 22 and 31, which are adenosine receptor antagonists. It is commonly known that adenosine receptor antagonists can be used against cardiovascular diseases and have e.g. an antiarteriosclerotic and antiinflammatory activity. This teaching is therefore novelty destroying for claims 11-14.

Documents D4-D8 are novelty destroying for claims 1,2,4,6. See passages/compounds mentioned in the search report.

Inventive step

It is credible that all compound of the present application have HM74A activity (see page 12, line 4-7). This was not obvious from D9 (closest prior art). The subject-matter of the present application is therefore inventive, but only insofar it is novel.

Clarity

Claims 1,11 are not clear, because these claims relate to compounds of formula (I) and **acceptable derivatives** thereof. Contrary to what is said in the description on page 7, lines 21-28, the scope cannot be determined by a skilled person without undue experimentation.

The definition of "aryl" on page 6, lines 29-33 is obscure. A 5-membered aryl group does not exist since cyclopentadienyl is (normally) not considered as an aromatic group. Furthermore an 8-membered bicyclic aromatic group does not exist.

Method of Treatment

For the assessment of the present claims 12,13 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims.

NS, can buy benzocyclobutenes
- cyclopentadienyl : correct element
considered as aromatic
group unless carrying a
negative charge

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/011376

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
✓ 2 X	WELLS, JACK N. ET AL: "Inhibition of separated forms of cyclic nucleotide phosphodiesterase from pig coronary arteries by 1,3-disubstituted and 1,3,8-trisubstituted xanthines" JOURNAL OF MEDICINAL CHEMISTRY, 24(8), 954-8 CODEN: JMCMAR; ISSN: 0022-2623, 1981, XP002169139 compound 4	1,2,4,5, 8-14
✓ 3 X	JACOBSON K A ET AL: "Effect of trifluoromethyl and other substituents on activity of xanthines at adenosine receptors" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol. 36, 1993, pages 2639-2644, XP002327691 ISSN: 0022-2623 compounds 22,31	11-14
✓ 4 X	CORSANO, STEFANO ET AL: "Structure-activity relationships in a series of 8-substituted xanthines as bronchodilator and A1-adenosine receptor antagonists" ARCHIV DER PHARMAZIE (WEINHEIM, GERMANY), 328(9), 654-8 CODEN: ARPMAS; ISSN: 0365-6233, 1995, XP008058976 compound 4	1,2,4,5
✓ 5 X	REGNIER G L ET AL: "New xanthine derivatives with potent and long lasting anti-bronchoconstrictive activity" EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 22, 1987, pages 243-250, XP002291236 ISSN: 0223-5234 compounds 39-41,45,47,49-52	1,2,4,5
✓ 6 X	EP 0 103 497 A (ADIR) 21 March 1984 (1984-03-21) compounds of formula (II) page 14; claim 14	1,2,4,5
✓ 7 X	BREDERECK, HELLMUT ET AL: "Syntheses in the purine series. XIII. The preparation of several xanthine-8-aldehydes" CHEMISCHE BERICHTE, 95, 403-13 CODEN: CHBEAM; ISSN: 0009-2940, 1962, XP002364488 compound III	1,2,4,5
✓ 8 X	US 2 879 271 A (KALLISCHNIGG ROLF) 24 March 1959 (1959-03-24) column 6, line 4	1,2,4
3	-/-	

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/084298 A (GLAXO GROUP LIMITED; FOORD, STEVEN, MICHAEL; PIKE, NICHOLAS, BRIAN; WI) 24 October 2002 (2002-10-24) cited in the application page 1 - page 6	1-16

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The preparations to be evaluated were administered orally to groups of six male Föllinsdorf rats of body weight range 160-200 g after the animals had fasted overnight. Compounds were dissolved or suspended in water by mixing in a mortar with 1 or 2 drops of Tween 80. Rats were dosed on a milligram per kilogram basis 1 h before measuring the thickness of both hind paws and the subplanter injection of 0.05 mL of carrageenan (Kraft Foods, lot 3F1.11), 1% w/v, in physiological saline. Three hours later the ensuing swelling was measured, and the percent inhibition of the edema formation was calculated by considering the edema in the control animals to be 100%. Paw thickness was measured using the avil and pla AASE Antinflammatory Screening Equipment (Roche, Basle) thickness-measuring transducer.

Passive Paw Anaphylaxis Test for Inhibitors of Immediate Hypersensitivity.²⁵ Sera containing heat-labile hemocytotropic antibodies for use in passive paw anaphylaxis (PPA) were raised in 150-200 g female brown Norway rats. Each rat was injected intraperitoneally with 0.5 mL of *Bordetella pertussis* vaccine (Commonwealth Serum Laboratories, Melbourne) containing 2×10^{10} organisms and intramuscularly with 2 mg of crystalline ovalbumin (OA) (Sigma, St. Louis, Grade VI, lot 67C-8036) in 0.5 mL of physiological saline.²⁶ Eleven days later, animals were bled and sera were recovered and tested for activity in passive cutaneous anaphylaxis (PCA) experiments in rats.²⁶

For PPA studies, groups of six to eight Oxford-Hooded (HO (PVG/C)) rats of approximately 200 g were used. Following the measurement of the thickness of both hind paws, rats were given subplanter injections in each paw of 0.05 mL of a suitable dilution of rat anti-OA serum. Two hours later, animals were injected iv in the tail with 0.3 mL of a 1% w/v solution of OA in physiological saline. Fifteen minutes after antigen challenge, paw swelling was measured using the AASE thickness-measuring transducer. Paws of control animals were injected with diluted antiserum, but these rats were not challenged with antigen before paw thicknesses were read. The mean increase in paw thickness in the antigen-challenged rats was obtained by deducting the mean figure obtained from control animals sensitized with diluted antiserum. Three other groups of control animals used in PPA studies were injected in the hind paws with saline, serum from immunized rats, or serum from rats injected with OA and *B. pertussis* but which gave a PCA titre of 0. Percent inhibition of the immediate allergic reaction was calculated by considering the swelling in the control paws to be 100%. Compounds tested for inhibition of PPA were given iv 5 min before antigen challenge.

Acknowledgment. The authors are indebted to the Physical Chemistry Department, Hoffmann-La Roche Inc., for providing IR, UV, NMR, and MS data, to Mrs. Karen May, Kilo Laboratory, for synthesis of the intermediate 2,3,5-tri-O-benzoyl- β -D-ribofuranosyl cyanide, and to Dr. M. Kramer, Immunotherapy Department, Hoffmann-La Roche Inc., for antiviral testing of 29 and 35.

(27) Winter, C. A.; Risley, E. A.; Nun, G. W. *Proc. Soc. Exp. Biol. Med.* 1963, 111, 544-547.

(28) Baldo, B. A.; Thomas, P. G.; Nelson, C. L.; Taylor, K. M. *Allergy*, in press.

(29) Mota, I. *Immunology* 1964, 7, 681-689.

(30) Ovary, Z. *Immunol. Methods* 1964, 252-284.

XP-002169139

P.D. 1981
p. 954-958 = 5

Inhibition of Separated Forms of Cyclic Nucleotide Phosphodiesterase from Pig Coronary Arteries by 1,3-Disubstituted and 1,3,8-Trisubstituted Xanthines

Jack N. Wells,* John E. Garst,¹ and George L. Kramer

Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232.
Received December 29, 1980

A series of xanthines with varied substituents in the 1, 3, and 8 positions were prepared in an attempt to understand the structure-activity relationship for alkylxanthines as inhibitors of two different forms of cyclic nucleotide phosphodiesterase. Polar substituents on the 1 or 3 position of the xanthine reduced the potency of the xanthines to inhibit both the calmodulin-sensitive and the "cyclic AMP specific" forms of phosphodiesterase. Polar substituents on the 8 position of the xanthine, other than a carboxylic acid, increased the potency to inhibit the calmodulin-sensitive form of phosphodiesterase, if they were capable of donating electrons to the xanthine nucleus. On the other hand, any substituent in the 8 position larger than H reduced the potency of the xanthines to inhibit the cyclic AMP specific form of phosphodiesterase. Topographical maps of the active sites of the two forms of phosphodiesterase are presented in summary.

Alkylxanthines (theophylline, caffeine, and 1-methyl-3-isobutylxanthine) are well known as inhibitors of cyclic nucleotide phosphodiesterase activity. We have demonstrated that modification of the xanthine structure can give rise to compounds that inhibit relatively selectively one of the two forms of phosphodiesterase found in pig coronary arteries (peak I and peak II).^{1,2} These enzymes appear to be representative of two of the major forms of phosphodiesterase, the calmodulin-sensitive form (peak I) and the "cyclic AMP specific" form (peak II), both of which are found in most mammalian cells. The cyclic

AMP specific form is characterized by its relative specificity for cyclic AMP as substrate and by its apparent negative cooperativity. The activity of this form of phosphodiesterase is not altered by calmodulin. The calmodulin-sensitive form has a lower apparent K_m for cyclic GMP ($\sim 1-3 \mu M$) than for cyclic AMP ($\sim 50-100 \mu M$). The V_{max} of this form of phosphodiesterase with cyclic AMP as substrate, however, is about 2-fold greater than is the V_{max} with cyclic GMP as substrate. Because of the lower K_m for cyclic GMP, this enzyme has been considered to be the cyclic GMP phosphodiesterase; indeed, this form seems to be almost solely responsible for cyclic GMP hydrolysis in pig coronary arteries. That is not to say, however, that the calmodulin-sensitive enzyme can not participate in the regulation of intracellular levels of cyclic AMP. At least in pig coronary arteries, total cyclic AMP phosphodiesterase activity at $1 \mu M$ substrate concentration is about equally divided between the calmo-

(1) Department of Animal Science, University of Illinois, Urbana, IL 61801.

(2) Garst, J. E.; Kramer, G. L.; Wu, Y. J.; Wells, J. N. *J. Med. Chem.* 1976, 19, 499.

(3) Kramer, G. L.; Garst, J. E.; Mitchel, S. S.; Wells, J. N. *Biochemistry* 1977, 16, 3318.

dulin-sensitive form and the cyclic AMP specific form. Thus, the calmodulin-sensitive form could contribute to cyclic AMP regulation, but whether it does or not in intact cells is not clear.

We have shown that these two enzyme forms also differ in their susceptibilities to inhibition by xanthines with varied alkyl substituents.^{2,3} Both forms appear to require the intact bicyclic ring system of xanthine^{4,5} and both are more potently inhibited by 1-methyl-3-isobutylxanthine (MIX) than by theophylline. It was shown, however, that alkyl or aralkyl substitution in the 7 position² or alkyl substitution in the 8 position³ of MIX gave compounds which were up to 35 times more potent as inhibitors of the calmodulin-sensitive form than of the cyclic AMP specific form. Conversely, substitution of larger alkyl groups for methyl in the 1 position of MIX gave compounds up to 5 times more potent as inhibitors of the cyclic AMP specific form than of the calmodulin-sensitive form. In this report, we present the results of structural modification of the xanthine nucleus in which the 1, 3, and 8 positions are substituted with various functionalities other than simple hydrocarbon groups. The agents were designed to extend the structure-activity relationships (SAR) of the xanthines as phosphodiesterase inhibitors and, at the same time, to obtain xanthines that are soluble enough in aqueous buffers to permit biological studies in the absence of organic solvents.

Results and Discussion

Theophylline has long been recognized as a phosphodiesterase inhibitor and is still often used in biological studies despite the commercial availability of more potent agents such as 1-methyl-3-isobutylxanthine and papaverine. With few exceptions, the xanthines listed in Table I are more potent than theophylline as inhibitors of either peak I (calmodulin sensitive) or peak II (cyclic AMP specific) activity. At least in part, this increased activity is attributable in substitution of isobutyl for methyl in the 3 position. Increased activity also results from allyl (14), methallyl (15), isoamyl,³ or benzyl³ substitution in this position. This increased potency appears to be due to hydrophobic binding, since the more polar hydroxyethyl (12) and acetoxyethyl (13) groups reduce potency to inhibit both peak I and peak II activities.

We have reported³ that for potent inhibition of either peak I or peak II activities, alkyl substituents rather than hydrogen are required at the 1 position. Potency to inhibit peak I activity is, however, reduced by substituting isoamyl or isobutyl for the 1-methyl group of MIX (Table I, compare IIX with MIX), and substitution of a carboxylic acid (10) in this position greatly reduces potency against either peak of activity. Conversion of 10 to the more lipophilic methyl ester (11) partially restores potency to inhibit both enzyme forms but presumably is still too polar to be well accommodated by the site which receives the isoamyl group of IIX.

We have also reported³ that alkyl substitution in the 8 position of MIX reduces potency to inhibit peak II activity but does not affect potency against peak I. An electron-withdrawing group (such as trifluoromethyl), however, reduces potency against both peaks of activity. As shown in Table I, the 8-(methylamino) (5) and 8-(dimethylamino) (6) functions both increased the potency of the MIX moiety to inhibit peak I and reduced the potency of the MIX moiety to inhibit peak II. These compounds (5 and 6) are, however, quite insoluble in aqueous buffers and,

in fact, are very poorly soluble in strong acid. Their usefulness is therefore limited, despite the fact that they are more than 50-fold more potent as inhibitors of peak I than as inhibitors of peak II activity. The lack of basic character of the amino group (i.e., lack of electron density on the nitrogen) and the increased potency to inhibit peak I activity indicate that electron-donating groups in this position increase potency against peak I. The carboxylic acid function (7) is the only 8-substituent that greatly reduces the potency of the 1-methyl-3-isobutylxanthine moiety to inhibit peak I phosphodiesterase activity. Potency against peak I is almost completely restored by conversion to the methyl ester (8). Thus, even relatively polar 8-substituents are well tolerated by peak I if they are not electron withdrawing, while any 8-substitution greatly reduces potency against peak II.

The data presented here and elsewhere^{2,3} can be summarized in the following manner. (1) Nonpolar substitution larger than methyl in the 3 position of the xanthine nucleus enhances potency against either peak of activity. It would appear from these data that the 3-substituent of the xanthine binds to a lipophilic site on both peak I and peak II. (2) Some substituent other than hydrogen is required at position 1 of the xanthine, and the size of this substituent has a great influence upon the potency of the xanthine to inhibit peak I but not peak II activity.³ The most straightforward interpretation of these data is that the site that accommodates the 1-substituent on the active site of peak I is limited in size, but the corresponding site on peak II can accommodate larger groups. The 1-substituent does not appear to contribute to the binding to peak II *per se*, because IIX and MIX are equipotent inhibitors of peak II activity. The sites on peak I and peak II apparently can not tolerate polarity, however, since 10 and 11 are poor inhibitors of either activity. (3) The sites on the two enzyme forms to which the xanthines bind apparently differ in their ability to tolerate bulk in the 8 position of the xanthine. Increased bulk in the 8 position and in the nearby 7 position² is well tolerated by the site of peak I, but the site on peak II cannot tolerate bulk in the 8 position. Substitution of groups ranging in size from methyl to *tert*-butyl³ or carbomethoxyethyl (8) onto the 8 position of the MIX molecule has little effect on the potency to inhibit peak I activity, whereas any of these substitutions drastically reduces the potency to inhibit peak II activity. In addition to the steric aspect of the 8-substituent, 8-substituents that withdraw electrons from the xanthine reduce the potency of the xanthine to inhibit either enzyme form.³ In contrast, agents that can donate electrons to the xanthine nucleus (5 and 6) increase the potency of the xanthine to inhibit peak I activity. The nature of this electronic effect on potency cannot be discerned from these data but may be related to alteration of hydrogen bonding, ring dipoles, or any combination of these and other parameters.

Using these generalizations and making a number of assumptions concerning the interaction of the two isozymes with their preferred cyclic nucleotide substrates, we have formulated working models of the active sites for peak I and peak II phosphodiesterases. The more potent xanthines are competitive inhibitors of cGMP hydrolysis by peak I phosphodiesterase activity (data not shown). Peak II activity, however, does not obey Michaelis-Menten kinetics and gives apparently negatively cooperative kinetic plots. For this reason, neither K_i values nor the type of inhibition can be determined by currently available methods. Nonetheless, it is reasonable to assume that the xanthines also bind to the hydrolytic site of peak II

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Table I. Preparative Data and Physical Properties of Substituted Xanthines and Inhibition of Separated Phosphodiesterases from Pig Coronary Arteries

compd	R ₁	R ₂	R ₃	mp °C	recryst solvent	yield %	formula	anal	I ₅₀ μM or % inhibn at 100 μM	
									peak I, cGMP	peak II, cAMP
1	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₂ CH=O(CH ₃) ₂	272-274 dec	EtOH		C ₁₄ H ₁₈ N ₄ O ₂	C, H	6.3 ± 0.9	21% ± 1
2	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₂ OCH ₃	197-198	EtOH	84	C ₁₄ H ₁₈ N ₄ O ₂	C, H	5.2 ± 1	212 ± 37
3	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₂ OH	216-217	H ₂ O	34	C ₁₄ H ₁₈ N ₄ O ₂	C, H	9.4 ± 2	283 ± 18
4	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₂ Cl	106- 108.5	EtOH	b	C ₁₄ H ₁₆ ClN ₄ O ₂	C, H	8.9 ± 2	28% ± 4
5	CH ₃	CH ₂ CH(CH ₃) ₂	NHCH ₃	285-287	EtOH	c	C ₁₄ H ₁₈ N ₄ O ₂	C, H	1.2 ± 0.1	39% ± 7
6	CH ₃	CH ₂ CH(CH ₃) ₂	N(CH ₃) ₂	284-285 dec	EtOH	d	C ₁₄ H ₁₈ N ₄ O ₂	C, H	1.6 ± 0.2	33% ± 3
7	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₂ CH ₂ CO ₂ H	270-272	EtOH	35	C ₁₆ H ₂₀ N ₄ O ₄	C, H	45 ± 8	12% ± 3
8	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₂ CH ₂ CO ₂ CH ₃	172-173	MeOH	e	C ₁₆ H ₂₀ N ₄ O ₄	C, H	9.8 ± 3	30% ± 4
9	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₂ CH ₂ CH ₂ OH	216-217	EtOH	60	C ₁₆ H ₂₀ N ₄ O ₄	C, H	11 ± 1	29% ± 3
10	CH ₂ CO ₂ H	CH ₂ CH(CH ₃) ₂	H	308 dec	EtOH	66	C ₁₄ H ₁₆ N ₄ O ₂	C, H	2.4% ± 2	1.9% ± 1
11	CH ₂ CO ₂ CH ₃	CH ₂ CH(CH ₃) ₂	H	172-174	benzene	f	C ₁₆ H ₂₀ N ₄ O ₄	C, H	38% ± 2	40% ± 1
12	CH ₃	CH ₂ CH ₂ OH	H	358 dec	EtOH	22	C ₁₄ H ₁₆ N ₄ O ₂	C, H	30% ± 1	23% ± 2
13	CH ₃	CH ₂ CH ₂ OCOCH ₃	H	197-198	EtOH	g	C ₁₆ H ₁₈ N ₄ O ₄	C, H	43% ± 5	27% ± 5
14	CH ₃	CH ₂ CH=CH ₂	H	210-211	EtOH	73	C ₁₄ H ₁₆ N ₄ O ₂	C, H	35 ± 3	52 ± 3
15	CH ₃	CH ₂ CH(CH ₃)=CH ₂	H	212-215	EtOH	69	C ₁₅ H ₁₈ N ₄ O ₂	h	10 ± 3	36 ± 3
16	CH ₂ CH ₂ CH(CH ₃) ₂	CH ₂ CH(CH ₃)=CH ₂	H	169-170	benzene	40	C ₁₆ H ₂₀ N ₄ O ₂	i	38% ± 4	42% ± 2
17 (1-methyl-3-isobutyl-8-oxaxanthine)				153-154	H ₂ O	72	C ₁₄ H ₁₈ N ₄ O ₂	C, H, N	38% ± 5	1.0% ± 1
MIX (1-methyl-3-isobutylxanthine) ^k									8.3 ± 1	15 ± 2
IX (1-isoxamyl-3-isobutylxanthine) ^k									86 ± 5	17 ± 3
theophylline									100 ± 9	190 ± 6

^a Yield from 1,3-disubstituted 6-nitroso-8-aminouracil ^b Prepared from 3 in 67% yield ^c Prepared from 1-methyl-3-isobutyl-8-bromoxanthine^h in 57% yield ^d Prepared from 1-methyl-3-isobutyl-8-bromoxanthine^h in 80% yield ^e Prepared from 7 in 78% yield ^f Prepared from 16 in 58% yield ^g Prepared from 12 in 53% yield ^h Structure established by hydrogenation to MIX ⁱ Structure established by hydrogenation to 1-isoxamyl-3-isobutylxanthine ^j I₅₀ values are the concentrations of inhibitor (μM) that inhibit by 50% the hydrolysis of 1 μM substrate ^k Values are means plus or minus standard errors of at least three determinations with three different preparations. ^l Data from ref 3

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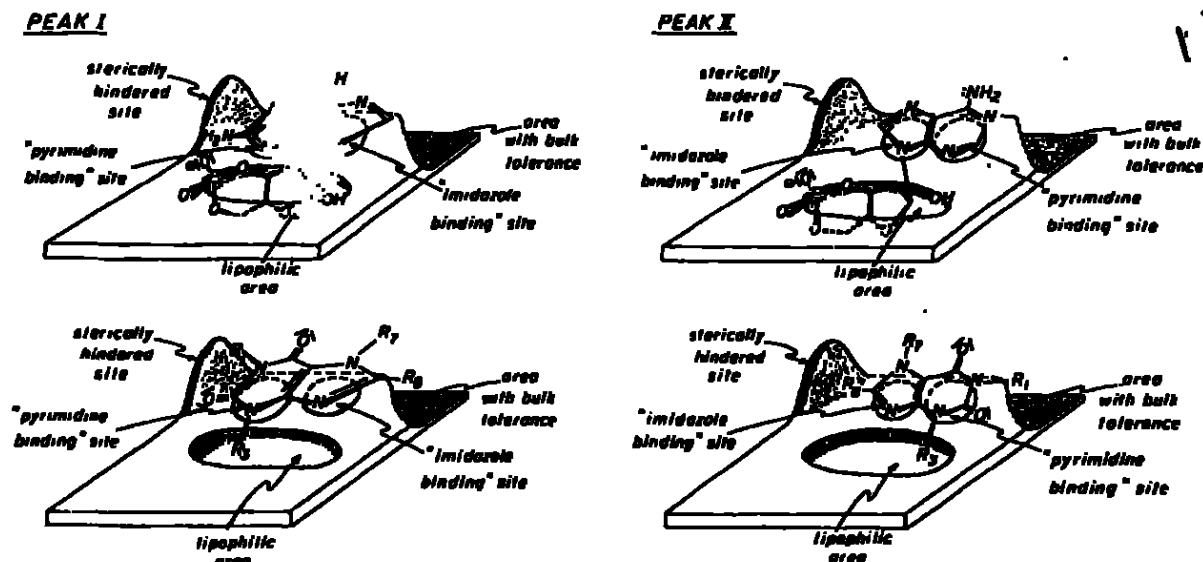


Figure 1. Hypothetical topographical map of the active sites of calmodulin-sensitive (peak I, left panel) and "cyclic AMP specific" (peak II, right panel) forms of phosphodiesterase. The upper panels represent the binding of the high-affinity substrates to the respective active sites, while the lower panels represent binding of a generalized xanthine to the active sites.

phosphodiesterase. If this is true, information concerning the nature of the active sites can be drawn both from a knowledge of the substrates and from the SAR of the inhibitors. Energy calculations indicate that the "syn" conformation of cyclic GMP is strongly preferred, while the "anti" conformation is somewhat preferred by cyclic AMP.⁵ To help account for the specificity of the two enzymes, it is assumed that the active sites accommodate their high-affinity substrates in its "preferred" conformation. Inspection of molecular models of the cyclic nucleotides in their "preferred" conformations will reveal that the ribose phosphate moiety which lies perpendicular to the plane of the purine ring is bisected by that plane into two very dissimilar sides. One side contains the 2' and 3' carbons, the charged phosphate moiety, and the 2'-hydroxyl and, thus, presents a relatively hydrophilic surface. The other side, however, contains only the 1', 4', and 5' carbons, the ether oxygen, and the 5'-ester oxygen and is relatively nonpolar. It is assumed that the catalytic sites of both peak I and peak II type phosphodiesterases bind to this more nonpolar portion of the ribose moieties of cyclic GMP and cyclic AMP, respectively. Some support for this assumption is found in a report that replacement of the charged phosphate group by an uncharged sulfate does not appreciably reduce the affinity of cyclic AMP for the catalytic site of phosphodiesterase.⁶ A further suggestion that the polar portion of the ribose-phosphate moiety is not required for binding to the catalytic sites is found in the high apparent affinity of some inhibitors for these sites. Papaverine, which lacks polar substituents, inhibits peak II hydrolysis of 1 μ M cyclic AMP by 50% at 3 μ M,⁷ and MIX, also lacking polar substituents, inhibits by 50% the hydrolysis of 1 μ M cyclic GMP by peak I at 6.3 μ M. Thus, the affinity of these inhibitors apparently approaches that of the high-affinity substrates. Replacement of the 3-methyl group of theophylline with larger and

more lipophilic groups (i.e., isobutyl to give MIX) dramatically increases the potency of the xanthine to inhibit both forms of phosphodiesterase. It seems reasonable to assume that, since both enzyme forms are involved in the hydrolysis of an essentially identical 3',5'-ribose cyclic phosphate, the ribose binding areas would be similar, perhaps having been conserved during evolution. Since variation of substituents at the 3 position of xanthine has similar effects on the potencies against both enzyme forms while variation of other positions has different effects, it is assumed that the 3-substituent of the xanthine is bound at the ribose binding sites. As shown in the upper panels of Figure 1, we envision this site as accommodating the nonpolar portion of the ribose phosphate while excluding the polar phosphate and hydroxyl groups of the cyclic nucleotides. Evidence indicates that the hydrolytic sites of peak I and peak II differ from each other essentially in the manner in which they accommodate the purine moiety of the cyclic nucleotides. These sites also differ in the manner in which they bind the xanthine ring of the inhibitors. The maps shown in Figure 1 account for these differences in part by reversing the orientation of the bicyclic ring binding sites. It is clear from the data in Table I and ref 3 that changes in the electronic properties of the xanthine ring have profound effects on inhibitory potencies against both peak I and peak II phosphodiesterases. The data do not indicate how these electronic effects are expressed. We have, therefore, depicted this binding in Figure 1 by labeling the sites as "pyrimidine" and "imidazole", simply to indicate the asymmetric nature of these interactions and the essentially reversed orientations in the peak I and peak II active sites. The sterically hindered sites to the left of the ring binding sites in Figure 1 account for the reduced affinity of xanthines with large 1-substituents for peak I and for the reduced affinity of xanthines with 7- or 8-substituents for peak II. The areas with bulk tolerance indicated to the right of the ring binding sites account for the ability of peak I to accommodate 7- or 8-substituted xanthines and for the ability of peak II to accommodate xanthines with 1-substituents larger than methyl.

Based upon this model, it would be predicted that increased alkyl bulk near the 6 position of the papaverine

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nucleus would lead to increased selectivity toward inhibition of peak II phosphodiesterase activity. The simple modification of replacing the 6-methoxyl with an isopropoxyl group does increase selectivity toward inhibition of peak II. This isopropoxyl analogue is one-half as potent as papaverine as an inhibitor of peak I activity but is 3-fold more potent than papaverine as an inhibitor of peak II activity (data not shown). Thus, while this working model of the topographical map of the two active sites is highly speculative, it does give some rational direction to the design of new phosphodiesterase inhibitors.

Experimental Section

Melting points were determined in open glass capillary tubes using a Laboratory Devices Mel-Temp and are reported uncorrected. Infrared spectra were obtained using a Perkin-Elmer Model 257 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a JOEL Model JNM-MH-100 spectrometer using tetramethylsilane as an internal standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. In all cases, spectral data were consistent with the proposed structures and elemental analyses were within $\pm 0.4\%$ of the calculated values.

Phosphodiesterase Assay. The compounds were assayed for inhibitory activity against the separated forms of phosphodiesterase from pig coronary arteries (peaks I and II). Enzyme preparation and assay procedures have been reported.^{12,13} Assays were performed with 1 μ M substrate at 30 °C for 30 min at enzyme dilutions that gave 10–20% hydrolysis of substrate in the absence of inhibitor. MIX (10^{-3} and 10^{-4} M) was included in each experiment to assure that the enzyme preparation was responding in a normal manner. The compounds were dissolved in 30% Me₂SO, and then 25 μ L of this solution was added to the assay tube (final volume was 250 μ L). All activities (including control and no-enzyme blank) were measured in the presence of 3% Me₂SO. Product accumulation was linear for at least 30 min under the conditions of the assay. Compounds that were sufficiently soluble in aqueous buffer were also tested in the absence of Me₂SO. The presence or absence of Me₂SO had no effect on the potency of the xanthines as phosphodiesterase inhibitors (data not shown). None of the compounds altered the efficiency of the nucleotidase step or subsequent steps in the assay. Concentrations of the compounds that inhibited by 50% the hydrolysis of 1 μ M substrate (I_{50}) were determined from concentration-percent inhibition curves, utilizing concentrations of the compounds from 10^{-7} to 10^{-4} M (or 10^{-2} to 10^{-5} M if the compound was sufficiently soluble). The more potent xanthines were competitive inhibitors of peak I phosphodiesterase, but K_i values are not reported, since the purpose of this study was to compare inhibition of the separated forms, and the apparently negatively cooperative behavior of peak II complicated the determination of K_i values with this enzyme. The presence or absence of calmodulin and/or calcium ion did not affect the activity of the compounds studied.

General Synthesis of Xanthines. The appropriate 1-substituted 6-aminouracil was prepared and alkylated following the procedure of Papasch and Schroeder¹⁴ to give the 1,3-disubstituted 6-aminouracil. The crude aminouracil (5–10 mmol) was suspended or dissolved by warming in 20% HOAC (25 mL). Sodium nitrite

(15 mmol) in H₂O was added dropwise to give the 5-nitroso-6-aminouracil as a red or purple precipitate. The crude 5-nitroso-6-aminouracil was dried in vacuo. The dry 5-nitroso-6-aminouracil was suspended in methanol and hydrogenated over PtO₂ at 50 psi of hydrogen in a Parr hydrogenator until the uptake of hydrogen had ceased. The catalyst was removed by filtration, and the volatiles were removed in vacuo. The allyl- and methyl-5-nitrosouracils were reduced to the diamines and isolated according to the method of Kramer et al.¹⁵ The crude diamino-uracil was dissolved in pyridine when reacted with about 1.5 equiv of an acid chloride or anhydride, but was reacted neat with excess hydroxyacetic acid, butyrolactone, or 97% formic acid. The mixture was heated to about 100 °C for 20 min. After the reaction was completed, the volatiles were removed and the residue was dissolved by heating under reflux in 10% sodium hydroxide for 20 min. The solution was cooled, extracted with benzene and/or methylene chloride to remove colored impurities, and then neutralized with HCl. The resulting precipitate was recrystallized from water or ethanol and water (Table I).

1-Methyl-3-isobutyl-8-aminoxanthine (17). 3-Methyl-1-isobutyl-5,6-diaminouracil (2.1 g, 10 mmol) was dissolved in 20 mL of 1 N HCl, and then 2 equiv of NaNO₂ in 15 mL of H₂O was added. The red precipitate that formed was collected by filtration and recrystallized from H₂O.

1-Methyl-3-isobutyl-8-(chloromethyl)xanthine (4). 1-Methyl-3-isobutyl-8-(hydroxymethyl)xanthine (3; 800 mg, 3.2 mmol) was heated at 100 °C with 10 mL of POCl₃ for 2.5 h. The volatiles were removed in vacuo. The residue was recrystallized from ethanol.

1-Methyl-3-isobutyl-8-(methylamino)xanthine (5). 1-Methyl-3-isobutyl-8-bromoxanthine² (1 g, 3.3 mmol) and 3 mL of 40% aqueous methylamine were dissolved in 3 mL of ethanol. The solution was heated at 130 °C for 12 h in a pressure tube. The volatiles were removed in vacuo and the residue was extracted with 10% sodium hydroxide. The basic solution was decolorized by treatment with charcoal and then acidified with HOAc. The resulting solid was recrystallized from ethanol.

1-Methyl-3-isobutyl-8-(dimethylamino)xanthine (6). 1-Methyl-3-isobutyl-8-bromoxanthine² (1 g, 3.3 mmol) was heated in a pressure vessel with 8 mL of 25% aqueous dimethylamine at 130 °C for 12 h. The resulting crystals were collected and recrystallized from ethanol.

1-Methyl-3-isobutyl-8-(carbamethoxyethyl)xanthine (8). 1-Methyl-3-isobutyl-8-(carboxyethyl)xanthine (7; 588 mg, 2 mmol) was dissolved in 200 mL of dry methanol, and 1 drop of concentrated H₂SO₄ was added. The solution was heated under reflux for 72 h. The volatiles were removed in vacuo to give a white residue that was recrystallized from methanol.

1-(Carbamethoxymethyl)-3-isobutylxanthine (11). 1-(Carboxymethyl)-3-isobutylxanthine (10) was esterified as described for 8 and recrystallized from benzene.

1-Methyl-3-(2-acetoxyethyl)xanthine (13). 1-Methyl-3-(2-hydroxyethyl)xanthine (12; 1 g, 4.8 mmol) was heated with 5 mL of acetyl chloride until most of the acetyl chloride had evaporated (about 1 h). H₂O (10 mL) was added, and the resulting precipitate was collected by filtration and recrystallized from ethanol.

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Effect of Trifluoromethyl and Other Substituents on Activity of Xanthines at Adenosine Receptors

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An aryl *p*-(trifluoromethyl) substituent increases the affinity of 1,3-disubstituted 8-phenylxanthines at A_{2A} -adenosine receptors, while having little effect on affinity at A_1 -adenosine receptors. In contrast, an aryl *p*-(trifluoromethyl) substituent has little effect on affinity of 3,7-disubstituted and 1,3,7-trisubstituted 8-phenylxanthines. An aryl *p*-sulfo substituent reduces affinity of all 8-phenylxanthines at A_1 - and A_{2A} -adenosine receptors. An 8-(trifluoromethyl) substituent markedly reduces affinity of 1,3-dialkylxanthines at both A_1 - and A_{2A} -adenosine receptors. In contrast, 8-(trifluoromethyl)caffeine retains affinity for A_{2A} -adenosine receptors, but does lose affinity for A_1 -adenosine receptors. 8-Bromo-, 8-acryl-, and 8-pent-1-enylcaffeinates are also selective for A_2 -adenosine receptors, while 8-cyclobutylcaffeine is nonselective. 8-(*trans*-2-(*tert*-butoxycarbonyl)vinyl)caffeine is 20-fold selective for A_{2A} vs A_1 receptors.

Structure-activity relationships for xanthines as adenosine receptor antagonists have been studied extensively over the last 2 decades.¹ High potency in xanthines that retain moderate water solubility and, hence, bioavailability and high selectivity for different classes of adenosine receptors have been the goals of such studies. Addition of an 8-phenyl substituent, particularly for 1,3-dipropylxanthines, yields extremely potent compounds with high selectivity for A_1 -adenosine receptors.²⁻¹⁰ Unfortunately, many of these 8-phenylxanthines have extremely low water solubility and hence poor bioavailability.¹⁴ The 8-cycloalkylxanthines proved to be more water soluble and to be both very potent and selective for A_1 -adenosine receptors. Xanthines with high selectivity for A_2 -adenosine receptors had not been forthcoming, although certain 1,3,7-trisubstituted xanthines exhibited modest selectivity for A_2 -adenosine receptors.^{14,15} Recently certain 8-styryl-1,3,7-trisubstituted xanthines were reported to be highly selective for A_2 -adenosine receptors.^{17,18}

The effects of aryl substituents on the potency and selectivity of 8-phenyltheophyllines have been analyzed. In one study on bovine brain A_1 receptors a quantitative structure-activity analysis for 45 aryl-substituted 8-phenyltheophyllines was presented.⁴ This study was preceded by a more limited exploration of structure-activity relationships for aryl substituted 8-phenyltheophyllines.⁵ Small electron-donating substituents at the ortho-position increased potency, while effects of para-substituents on activity were not readily correlated with electronic or steric effects or lipophilicity. Meta-substituents tended to decrease activity. Polar substituents such as *p*-sulfo or *p*-carboxy that are electron-withdrawing reduced potency at both A_1 - and A_2 -adenosine receptors, while conferring high water solubility.⁴ Such xanthines have proven useful as peripheral adenosine receptor antagonists.^{14,19}

The effects of xanthine substituents other than aryl at the 8-position have not been studied extensively. 8-Cycloalkyl-1,3-dialkylxanthines are potent and selective antagonists for A_1 -adenosine receptors.^{9,10,11,12} 8-Cycloalkyl-1,3,7-trialkylxanthines¹¹ and 8-styryl-1,3,7-trialkylxanthines^{17,18} are selective antagonists for A_2 -adenosine receptors.

The present study examines the effects at adenosine receptors of the electron-withdrawing trifluoromethyl group, either in the 8-position or in the para-position of 8-phenylxanthines and various other groups, such as halo, alkyl, or vinyl at the 8-position. Increases in potency at A_2 -adenosine receptors occur on aryl *p*-(trifluoromethyl) substitution of certain 8-phenylxanthines. Various 8-substituted caffeines, including 8-(trifluoromethyl)caffeine and 8-vinylcaffeinates are somewhat selective for A_2 -adenosine receptors.

Chemistry

Synthesis of xanthines substituted at 1-, 3-, 7-, and 8-positions (Tables I and II) involved the acylation of appropriate 5,6-diaminouracil to afford 5-(arylamino)-6-aminoouracils (e.g. 40-44), which were then cyclized to the 8-arylxanthines (2, 6, 8, 9, 11), and 8-cycloalkylxanthine (25), or an 8-vinylxanthine (26) by sodium hydroxide. The 8-(*p*-sulfonamidophenyl)xanthine 11 was converted to the corresponding 8-(*p*-sulfonylphenyl)xanthine 10 with NaNO_2 in trifluoroacetic acid. Certain 1,3,7-trisubstituted 8-phenylxanthines (13, 16-19) were prepared by alkylation of the appropriate disubstituted 8-phenylxanthine. 8-(Trifluoromethyl)-1,3-di-*n*-propylxanthine (29) and the corresponding theophylline derivative (22) were obtained directly from the diaminoouracil after refluxing with trifluoroacetic acid and subsequent cyclization under basic conditions. An 8-acrylcaffeine derivative (28) was synthesized from 8-bromocaffeine and *tert*-butyl acrylate using the Heck reaction (Scheme 1).²⁰ The *tert*-butyl ester was cleaved in trifluoroacetic acid to provide a carboxylic congener (28).

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Table I. Effect of Aryl Trifluoromethyl and Sulfide Substituents on Activity of 8-Phenylxanthines at Adenosine Receptors of Rat Brain Membranes

no.	xanthine substituents	K_i (μ M) or % inhibitor ^a	
		A_1 -binding, [PH]PIA	A_2 -binding, [PH]CGS 21060
1	1,3-dimethyl-8-phenyl	0.078 $\pm$ 0.013	0.07 $\pm$ 0.08
2	1,3-dimethyl-8-[p-(trifluoromethyl)phenyl]	0.15 $\pm$ 0.03	0.31 $\pm$ 0.03
3	1,3-dimethyl-8-(p-sulfophenyl)	1.0 $\pm$ 0.33	8.8 $\pm$ 0.7
4	1,3-dipropyl-8-phenyl	0.010 $\pm$ 0.004	0.14 $\pm$ 0.01
5	1,3-dipropyl-8-[p-(trifluoromethyl)phenyl]	0.0078 $\pm$ 0.0005	0.043 $\pm$ 0.004
6	1,3-dipropyl-8-(p-sulfophenyl)	0.14 $\pm$ 0.04	0.79 $\pm$ 0.03
7	1,3-dipropyl-8-(p-sulfonamidophenyl)	0.0048 $\pm$ 0.0011	0.075 $\pm$ 0.009
8	3,7-dimethyl-8-phenyl	3.4 $\pm$ 0.3	13 $\pm$ 1
9	3,7-dimethyl-8-[p-(trifluoromethyl)phenyl]	8.7 $\pm$ 0.3	10% (20 μ M)
10	3,7-dimethyl-8-(p-sulfophenyl)	1.9% (250 μ M)	0% (250 μ M)
11	3,7-dimethyl-8-(p-sulfonamidophenyl)	33% (250 μ M)	42 (250 μ M)
12	8-phenyl-1,3,7-trimethyl	18 $\pm$ 3	18 $\pm$ 1
13	8-[p-(trifluoromethyl)phenyl]-1,3,7-trimethyl	17 $\pm$ 3	18 $\pm$ 1
14	8-(p-sulfophenyl)-1,3,7-trimethyl	8.3% (250 μ M)	30 $\pm$ 1
15	1-allyl-3,7-dimethyl-8-phenyl	0.0 $\pm$ 0.1	13 $\pm$ 1
16	1-allyl-3,7-dimethyl-8-[p-(trifluoromethyl)phenyl]	0.7 $\pm$ 0.0	11 $\pm$ 1
17	1-allyl-3,7-dimethyl-8-(p-sulfophenyl)	33% (250 μ M)	37 $\pm$ 4
18	1,7-diallyl-8-methyl-8-phenyl	0.08 $\pm$ 0.15	7.7 $\pm$ 0.6
19	7-allyl-1,3-dipropyl-8-[p-(trifluoromethyl)phenyl]	2.0 $\pm$ 0.1	4.3 $\pm$ 0.5

^a Values are means $\pm$ SEM ($n = 3$) or are percent inhibition at highest concentration tested. The highest concentration is given in parentheses. In some cases higher concentrations could not be tested because of solubility.

Table II. Effect of 8-(Trifluoromethyl), 8-Methyl, 8-Halo, and 8-Vinyl Substituents on Affinity of Xanthines at Adenosine Receptors of Rat Brain Membranes

		K_i (μ M) or % inhibitor ^a	
no.	substituents	A_1 binding, [TH]PIA	A_2 -binding, [PH]CGS 21060
Substituted Theophylline			
20	none	14 $\pm$ 3	8.0 $\pm$ 1.3
21	8-methyl	8.8 $\pm$ 3.0	34% (250 μ M)
22	8-(trifluoromethyl)	0% (250 μ M)	0% (250 μ M)
23	8-chloro	39% (250 μ M)	34 (100 μ M)
24	8-bromo	10.3 $\pm$ 0.3	56% (100 μ M)
25	8-cyclobutyl	0.19 $\pm$ 0.01	2.6 $\pm$ 0.1
26	8-pent-1-enyl	0.23 $\pm$ 0.05	0.44 $\pm$ 0.03
Substituted Xanthine			
27	3-isobutyl-1-methyl	7 $\pm$ 21	5.8 $\pm$ 1.1
28	8-bromo-3-isobutyl-1-methyl	65 $\pm$ 21	10% (100 μ M)
29	1,3-dipropyl	0.7 $\pm$ 0.3	2.8 $\pm$ 0.2
30	1,3-dipropyl-8-methyl	0.6 $\pm$ 0.1	2.8 $\pm$ 0.2
31	1,3-dipropyl-8-(trifluoromethyl)	4.5% (30 μ M)	4.5% (30 μ M)
Substituted Caffeine			
32	none	20 $\pm$ 6	23 $\pm$ 3
33	8-methyl	4.8 $\pm$ 0.3	7.5 $\pm$ 0.8
34	8-(trifluoromethyl)	8% (100 μ M)	29 $\pm$ 3
35	8-bromo	48 $\pm$ 5	13 $\pm$ 1
36	8-cyclobutyl	30 $\pm$ 2	19 $\pm$ 1
37	8-pent-1-enyl	3.1 $\pm$ 0.4	1.9 $\pm$ 0.1
38	8-(trans-2-carboxyvinyl)	3% (100 μ M)	43 $\pm$ 7
39	8-[trans-2-(tert-butylcarboxy)vinyl]	13 $\pm$ 3	0.81 $\pm$ 0.09

^a Values are means $\pm$ SEM ($n = 3$) or are percent inhibition at highest concentration tested. See legend of Table I.

Results and Discussion

1,3-Dimethyl-8-phenylxanthine (8-phenyltheophylline, 1) is widely used as a potent adenosine receptor antagonist, which is somewhat selective ($\sim$ 13-fold) for A_1 -receptors. The presence of an electron-withdrawing *p*-(trifluoromethyl) substituent has little effect on affinity at rat brain A_1 -receptors, but increases activity at rat brain A_2 -receptors by about 5-fold (Table I), resulting in a potent, but now nonselective, antagonist (3).

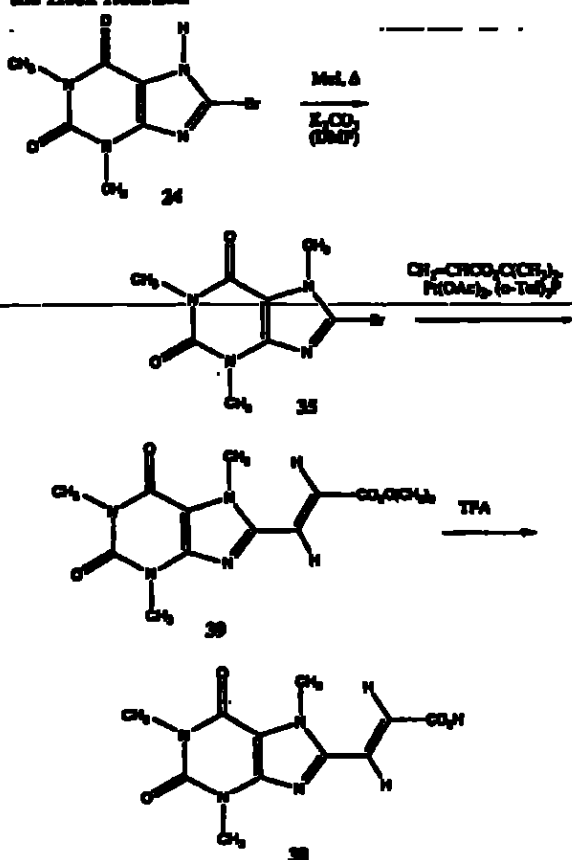
The effect of aryl substituents on the activity of 1,3-dimethyl-8-phenylxanthines at A_1 -receptors has been studied.^{3,4} Electron-donating substituents (hydroxy, amino) in the ortho-position appear to increase activity at A_1 -receptors.⁴ Correlations of activity with electronic or steric effects or lipophilicity of para-substituents were not clear.⁴ At rat striatal A_2 -receptors *p*-amino, *p*-chloro, and

p-methoxy substituents all increased the potency of 1,3-dimethyl-8-phenylxanthine by about 2-fold.³ At both A_1 - and A_2 -receptors, an anionic para-substituent (COO^- , $-\text{SO}_3^-$) markedly decreased potency.^{4,9} In the present study, a *p*-(trifluoromethyl) group had the same effects on activity for 1,3-dipropyl-8-phenylxanthine (5 vs 4) as it had for 8-phenyltheophylline (2 vs 1), namely little effect on affinity at A_1 -receptors and about a 3-fold increase in affinity at A_2 -receptors (Table I).

3,7-Dimethyl-8-phenylxanthine, 8, proved to be relatively weak, compared to 1,3-disubstituted 8-phenylxanthines (e.g. 1) as an adenosine receptor antagonist (Table I). A *p*-(trifluoromethyl) substituent decreased affinity at A_2 -receptors (9 vs 8).

1,2,7-Trisubstituted 8-phenylxanthines, such as 8-phenylcaffeine, 12, are relatively weak, compared to 1,3-

Scheme 1. Synthesis of 8-Acrylxanthine Derivatives via the Heck Reaction



disubstituted 8-phenylxanthines, as adenosine receptor antagonists.¹¹ The effects of aryl substituents on activity have not been probed. In the present study, an electron-withdrawing *p*-(trifluoromethyl) group had little effect on affinity of either 8-phenyl-1,3,7-trimethylxanthine (8-phenylcaffeine) (12 vs 13) or of 1-allyl-3,7-dimethyl-8-phenylxanthine (16 vs 15). A *p*-(trifluoromethyl) substituent did decrease affinity of 1,7-diallyl-3-methyl-8-phenylxanthine (19 vs 18) at A_1 -receptors by about 4-fold.

Among the 8-[*p*-(trifluoromethyl)phenyl]xanthines in this study, no compounds were developed that have any particular advantage in A_1 -selectivity or potency over the corresponding 8-phenylxanthine, and the trifluoromethyl group would be expected to cause an undesirable reduction in water solubility.

In contrast, the high water solubility conferred by an aryl sulfo substituent has made 1,3-dimethyl-8-(*p*-sulfophenyl)xanthine [8-(*p*-sulfophenyl)theophylline, 3] and 1,3-dipropyl 8-(*p*-sulfophenyl)xanthine, 6, useful research tools since they do not penetrate into cells²⁰ or cross the blood-brain barrier.²¹ Both are relatively weak, compared to the parent 8-phenylxanthines, as adenosine receptor antagonists, and both (3 and 6) are only modestly A_1 -selective (Table I). An aryl sulfonamide substituent does not decrease activity of 1,3-dipropyl-8-phenylxanthine (7 vs 8). The resulting xanthine, 7, is nearly as A_1 -selective as the parent xanthine and might be more water soluble. In the 3,7-disubstituted and 1,3,7-trisubstituted 8-phenylxanthines, an aryl sulfo (or sulfonamide) substituent

(10, 14, 17) also markedly reduces affinity at adenosine receptors. The 8-(*p*-sulfophenyl)-1,3,7-trimethylxanthine [8-(*p*-sulfophenyl)caffeine, 14] is somewhat selective for the rat brain A_2 -receptor, compared to the rat brain A_1 -receptor (Table I). However, in other A_1 - and A_2 -receptor comparisons the selectivity for 14 for A_2 -receptors was not apparent.¹¹

Effects of substituents other than aryl, cycloalkyl, or alkyl at the 8-position on activity of xanthines at adenosine receptors have received little attention.²² An 8-methyl group (21, 20, 23) slightly increases the affinity of theophylline, 1,3-dipropylxanthine, and caffeine at rat brain A_1 -receptors, while either reducing (21), increasing (23), or having little effect (20) on affinity at rat brain A_2 -receptors (Table I). An 8-(trifluoromethyl) substituent (22, 21) nearly eliminates affinity of theophylline and 1,3-dipropylxanthine at both A_1 - and A_2 -receptors. 3-Isobutyl-1-methyl-8-(trifluoromethyl)xanthine was previously reported to have very low potency at A_1 - and A_2 -receptors.¹² An 8-(trifluoromethyl) substituent (24) also reduces the affinity of caffeine at A_1 -receptors, while having virtually no effect on its affinity at A_2 -receptors (Table II). 8-Halo substituents (Cl, Br) markedly reduce affinity of 1,3-disubstituted xanthines at adenosine receptors with the exception of 8-bromotheophylline (24), which has a slightly higher affinity at A_1 receptors than theophylline (Table II). 8-Bromocaffeine, 24, has a slightly higher affinity than caffeine at both A_1 and A_2 receptors. An 8-cyclobutyl substituent (25) markedly increases affinity of theophylline for adenosine receptors (Table II), as was the case for 8-cyclopentyl- and 8-cyclohexyltheophyllines,^{23,24} but the degree of A_1 -selectivity (15-fold) is not as large as for 8-cyclopentyltheophylline.⁹ 8-Pent-1-enyltheophylline, 26, is a potent antagonist, but unlike the 8-cycloalkyltheophyllines, is not selective for A_1 receptors (Table II). In contrast, an 8-cyclobutyl substituent (26) has virtually no effect on the affinity of caffeine at adenosine receptors, and the 8-pent-1-enylcaffeine, 27, shows a slight selectivity (8-fold) for A_2 receptors (Table II). In view of the marked A_2 -selectivity of 8-styrylcaffeines,^{17,18} two 8-acryl derivatives of caffeine, namely the water soluble 8-(*trans*-2-carboxyvinyl)caffeine, 28, and 8-[*trans*-2-(*tert*-butyloxycarbonyl)vinyl]caffeine 29 were prepared. Both were A_2 -selective (Table II). The 20-fold selectivity of 29 suggests that the functionalized congener approach⁹ could be applied to such caffeine derivatives to develop a range of selective A_2 -antagonists, e.g. esters and other derivatives of the carbonyl congener 28. The A_2 -selectivity of 8-vinylcaffeine derivatives demonstrates that a phenyl group, as in the structurally similar, A_2 -selective 1,3,7-trialkyl-8-styrylxanthines,^{17,18} is not required for this selectivity. The Pd-catalyzed Heck reaction²⁵ represents a new approach to 8-substitution, especially 8-vinyl substitution, of xanthines.

The present study indicates that the presence of 8-substituents, including a *p*-(trifluoromethyl) group on an 8-phenyl and 8-methyl, 8-(trifluoromethyl), 8-halo, 8-cycloalkyl, 8-pent-1-enyl and 8-acryl groups, can have markedly different effects on affinity for adenosine receptors when the xanthine is a caffeine analog (1,3,7-substituted) rather than a theophylline analog (1,3-substituted). Such differences suggest either that caffeine analogs bind differently than theophylline analogs to adenosine receptors or that the conformational effects of 7- and 8-position substituents are interdependent. 8-Substituted caffeine analogs, including the 8-styryl com-

pounds^{7,10} and the present 8-acryl compounds (Table II), appear likely to provide long-sought potent and selective A₂-receptor antagonists.

Experimental Section

Chemistry. Compounds 1, 3, 4, 6, 7, 14 were synthesized as reported¹⁴⁻¹⁶ or were obtained from Research Biochemicals International (Natick MA). CHN analysis ($\pm 0.4\%$ acceptable) were obtained for new compounds synthesized (3, 5, 8, 9-11, 13, 15-19, 22, 25, 26, 29, 32, 34, 37-39, 40-44). All melting points were determined without correction using a Gallenkamp apparatus. UV/vis spectra were done with a Perkin-Elmer, Lambda 5 spectrometer; λ_{max} is reported in nanometers (log ϵ). ¹H-NMR spectra were determined with a Bruker WM-250 and are reported δ in ppm relative to TMS as internal standard.

8-[4-(Trifluoromethyl)phenyl]-1,3-dimethylxanthine (2). 6-Amino-8-[(4-(trifluoromethyl)benzoyl)amino]-1,3-dimethyluracil (40, 25 g) was refluxed in 40 mL of 1 N NaOH and 10 mL of EtOH for 1 h. The hot solution was acidified with AcOH, and the precipitate was collected after cooling. Recrystallization from MeOH gave 1.88 g (71%) of colorless crystals. Mp: $>350^\circ\text{C}$. UV (MeOH): 225 sh (4.35), 238 (4.32), 294 sh (3.70), 318 (4.32).

8-[4-(Trifluoromethyl)phenyl]-1,3-di-*n*-propylxanthine (8). 6-Amino-8-[(4-(trifluoromethyl)benzoyl)amino]-1,3-di-*n*-propyluracil (41, 2.0 g) was refluxed in 50 mL of 2 N NaOH and 10 mL of EtOH for 1 h. The hot solution was acidified with AcOH, and after cooling, the precipitate was collected, washed with water, and dried at 100°C to give 1.45 g (78%) of chromatographically pure crystals. Mp: $250-261^\circ\text{C}$. UV (MeOH): 232 (4.32), 238 (4.30), 318 (4.30).

3,7-Dimethyl-8-(4-sulfophenyl)xanthine (6). 6-Amino-8-benzoyl-*N*-(methylamino)-1-methyluracil (43, 17 g, 70 mmol) was refluxed in 150 mL of 2 N NaOH and 50 mL of EtOH for 1 h. The hot solution was diluted with 200 mL of H₂O and then acidified with AcOH to give a colorless precipitate. After drying at 100°C , 15.6 g (88%) of a chromatographically pure solid (mp: $>300^\circ\text{C}$) was obtained. UV (MeOH): 205 (4.32), 229 (4.23), 293 (4.11).

8-[4-(Trifluoromethyl)phenyl]-3,7-dimethylxanthine (9). 6-Amino-8-(4-(trifluoromethyl)benzoyl)-*N*-(methylamino)-1-methyluracil (42, 6.54 g, 30 mmol) was refluxed in 100 mL of 2 N NaOH and 30 mL of EtOH for 1 h. The hot solution was acidified with AcOH, and after cooling, the solid was collected to give 5.8 g (86%) of chromatographically pure crystals. Mp: $304-305^\circ\text{C}$. UV (MeOH): 205 (4.30), 223 (4.31), 230 sh (4.30), 303 (4.18).

3,7-Dimethyl-8-(4-sulfophenyl)xanthine (10). 3,7-Dimethyl-8-(4-sulfamidophenyl)xanthine (11, 16.75 g, 0.05 mol) was dissolved in 300 mL of trifluoroacetic acid, and then at room temperature a solution of 5 g of NaNO₂ in 10 mL of water was added dropwise with stirring. The reaction was diluted with 200 mL of H₂O and then stirred for another 3 h to form a crystalline precipitate. The chromatographically pure product was dried to provide 16.1 g (95%) of a crystalline powder. Mp: $>300^\circ\text{C}$. UV (pH 2): 223 sh (4.32), 235 (4.33), 297 (4.34).

3,7-Dimethyl-8-(4-sulfamidophenyl)xanthine (11). 6-Amino-8-(4-sulfamidobenzoyl)-*N*-(methylamino)-1-methyluracil (44, 35 g, 71 mmol) was refluxed in 150 mL of 2 N NaOH and 50 mL of MeOH for 1 h. The hot solution was diluted with 200 L of H₂O and then acidified with AcOH to form a colorless precipitate. After drying, 31.4 g (90%) of a chromatographically pure, crystalline powder (mp: $>300^\circ\text{C}$) was obtained. UV (pH 1): 228 (4.31), 235 sh (4.31), 301 (4.31).

8-[4-(Trifluoromethyl)phenyl]-1,3,7-trimethylxanthine (12). 8-[4-(Trifluoromethyl)phenyl]-1,3-dimethylxanthine (2, 1.63 g, 5 mmol) was treated in 100 mL of DMF with 3 g of K₂CO₃ and 5 mL of methyl iodide for 3 h with stirring at room temperature. The inorganic salts were filtered off, the filtrate was evaporated to dryness, and the residue was treated with 15 mL of water. The crystalline solid was recrystallized from MeOH to give 1.56 g (92%) of colorless crystals. Mp: $180-182^\circ\text{C}$. UV (MeOH): 203 (4.30), 224 (4.30), 303 (4.18).

1-Allyl-3,7-dimethyl-8-phenylxanthine (15). A solution of 3,7-dimethyl-8-phenylxanthine (5, 15 g, 50 mmol) in 500 mL of DMF was treated with 45 g of K₂CO₃ and allyl iodide (14.4 g, 80 mmol) at 80°C for 4 h with stirring. After cooling, the inorganic

salts were filtered off, and the filtrate was reduced in volume by evaporation in vacuo. The residue was treated with 200 mL of H₂O to give a colorless solid, which on recrystallization from H₂O/EtOH gave 14.8 g (88%) colorless crystals. Mp: $177-178^\circ\text{C}$. UV (MeOH): 220 (4.34), 233 (4.32).

1-Allyl-8-[4-(trifluoromethyl)phenyl]-3,7-dimethylxanthine (16). A solution of 8-[4-(trifluoromethyl)phenyl]-3,7-dimethylxanthine (5, 1.63 g, 5 mmol) in 100 mL of DMF was treated with 3 g of K₂CO₃ and allyl iodide (1.5 g) at room temperature for 3 h with stirring. The inorganic salts were filtered off, the filtrate was evaporated to dryness, and the residue was treated with 50 mL of H₂O to give a colorless solid. Recrystallization from H₂O/EtOH gave 1.55 g (85%) of colorless crystals. Mp: $180-182^\circ\text{C}$. UV (MeOH): 204 (4.34), 223 (4.32), 230 sh (4.30), 303 (4.17).

1-Allyl-3,7-dimethyl-8-(4-sulfophenyl)xanthine (17). A suspension of 16 (16.7 g, 0.05 mol) in 300 mL of DMF was treated with 5 g of sodium hydride and 25 g of allyl iodide at room temperature for 3 h. The inorganic salts were filtered off, and the filtrate was evaporated to dryness. The residue was treated with 100 mL of acetone, to give a solid, which was recrystallized from 50 mL of 5 N HCl to give 13.6 g (73%) of colorless crystals. Mp: $>300^\circ\text{C}$. UV (pH 12): 203 (4.43), 235 (4.36), 295 (4.28).

1,7-Diallyl-3-methyl-8-phenylxanthine (18). A mixture of 3-methyl-8-phenylxanthine (243 g, 10 mmol) and 9 g of K₂CO₃ was treated in 100 mL of DMF with allyl iodide (4 g) at 80°C with stirring for 3 h. The inorganic salts were filtered off, the filtrate was evaporated to dryness, and the residue was recrystallized from H₂O/MeOH to give 2.4 g (75%) of colorless crystals. Mp: $104-106^\circ\text{C}$. UV (MeOH): 204 (4.44), 230 (4.33), 293 (4.20).

7-Allyl-8-[4-(trifluoromethyl)phenyl]-1,3-di-*n*-propylxanthine (19). A mixture of 8-[4-(trifluoromethyl)phenyl]-1,3-di-*n*-propylxanthine (8, 1.63 g, 4.3 mmol) and 3 g of K₂CO₃ were treated in 100 mL of DMF with 3 g of allyl iodide at room temperature with stirring for 3 h. The inorganic salts were filtered off, the filtrate was evaporated to dryness, and the residue was treated with 50 mL of water. The precipitate was recrystallized from H₂O/EtOH to give 1.4 g (80%) of colorless crystals. Mp: $63-65^\circ\text{C}$. UV (MeOH): 205 (4.30), 223 (4.32), 230 sh (4.30), 303 (4.11).

8-(Trifluoromethyl)theophylline (23). 5,6-Dimino-1,3-dimethyluracil (4.5 g, 0.05 mol) was refluxed in 50 mL of trifluoroacetic anhydride for 30 min. The reaction mixture was evaporated to dryness and the residue treated with 50 mL of 2 N NaOH for 30 min under reflux. The hot solution was acidified to pH 3 and the precipitate collected after cooling. The colorless crystals were recrystallized from a mixture of H₂O/EtOH (2:2) to give 8.07 g (73%) of colorless crystals. Mp: $289-270^\circ\text{C}$. UV (pH 12): 216 (4.03), 235 sh (3.61), 274 (4.06). ¹H NMR (DMSO-*d*₆): δ 3.4 (s, 3H, NCH₃), 3.51 (s, 3H, NCH₃), 12.8 (s, 1H, NH).

8-Cyclobutyl-1,3-dimethylxanthine (26). Compound 26 was made from cyclobutane acid according to the general procedure A given in ref 18 in 28% yield. Mp: $>300^\circ\text{C}$ dec. ¹H NMR (DMSO-*d*₆): δ 1.8-2.1 (m, 2H, CH₂, 3 of Bu), 2.3 (m, 4H, CH₂, C2 and C4 of Bu), 3.23 (s, 3H, NCH₃), 3.43 (s, 3H, NCH₃), 3.68 (m, 1H, Cl of Bu). MS (EI): *m/z* 234, 205.

trans-8-Pent-1-enyl-1,3-dimethylxanthine (28). Compound 28 was made from trans-8-benzene acid according to the general procedure A given in ref 18 in 8% yield and recrystallized from DMF/water. Mp: $214-223^\circ\text{C}$ dec. ¹H NMR (DMSO-*d*₆): δ 0.91 (t, 3H, CH₃), 1.45 (m, 2H, CH₂), 2.30 (s, 3H, CH₃, J = 7 Hz), 3.23 (s, 3H, NCH₃), 3.43 (s, 3H, NCH₃), 6.27 (d, 1H, J = 16 Hz), 6.81 (dt, 1H, J = 16 Hz, J = 7 Hz). MS (CI NH₃): *m/z* 268 (MNH₃⁺, base), 241 (MH⁺).

8-(Trifluoromethyl)-1,3-di-*n*-propylxanthine (29). 5,6-Dimino-1,3-di-*n*-propyluracil (4 g, 0.015 mol) was refluxed in 40 mL of trifluoroacetic anhydride for 1 h. The reaction mixture was evaporated to dryness, the residue was treated with 50 mL of 2 N NaOH under reflux for 30 min, and then acidified to pH 4, and the crystalline precipitate was collected. Recrystallization from H₂O/EtOH gave 4.3 g (80%) of colorless crystals. Mp: 172°C . UV (pH 12): 216 (4.06), 235 sh (3.58), 275 (4.06).

8-(Trifluoromethyl)caffeine (32). 8-(Trifluoromethyl)theophylline (22, 2.48 g, 0.01 mol) was dissolved in DMF (50 mL) and then K₂CO₃ (7.5 g, 0.05 mol) and methyl iodide (2.11 g, 0.015 mol) added and stirred for 2 h at room temperature. The insoluble

Inorganic salts were filtered off, the filtrate was evaporated to dryness, and the residue was recrystallized from $H_2O/EtOH$ (4:1) to give 2.18 g (88%) of colorless crystals. Mp: 131–133 °C. UV (MeOH): 207 (4.39), 232 sh (3.64), 281 (3.92). 1H NMR (DMSO- d_6): δ 4.12, 3.55, 3.38 (each s, 3H, NCH_3). MS (EI): m/e 282, 193, 177.

8-Cyclohexyl-1,3,7-trimethylxanthine (36). Compound 36 was made from compound 28 according to procedure B given in ref 18. It was recrystallized from DMF/water (72% yield). Mp: 181–183 °C. MS (EI): m/e 248, 230.

trans-8-Pent-1-enyl-1,3,7-trimethylxanthine (37). Compound 37 was made from compound 28 according to procedure B given in ref 18. It was recrystallized from DMF/water (64% yield). Mp: 143–143 °C. 1H NMR (DMSO- d_6): δ 0.93 (t, 3H, CH_3), 1.80 (m, 2H, CH_2), 2.28 (q, 2H, $J = 7$ Hz), 3.31 (s, 3H, NCH_3), 3.41 (s, 3H, NCH_3), 3.59 (s, 3H, NCH_3), 5.57 (d, 1H, $J = 16$ Hz), 6.88 (dt, 1H, $J = 16$ Hz, $J = 7$ Hz). MS (EI): m/e 282, 247 (M – CH_2 , base).

8-(trans-3-Carboxyvinyl)-1,3,7-trimethylxanthine (38). Compound 38 (76 mg, 236 μ mol) was dissolved in 3 mL of trifluoroacetic acid and stirred for 1 h. After evaporation, the residue was triturated with ether to provide the pure product (55 mg, 58% yield). Mp: 378 °C dec. 1H NMR (DMSO- d_6): δ 3.37 (s, 3H, NCH_3), 3.44 (s, 3H, NCH_3), 4.02 (s, 3H, NCH_3), 6.78 (d, 1H, $J = 16.4$ Hz), 7.55 (d, 1H, $J = 16.4$ Hz), 8.4 (br s, 1H, COOH). MS (CI NH_3): m/e 285 (MH $^+$).

Alternately compound 38 was prepared from 39 in DMF/water (1:1) solution by saponification with sodium hydroxide in 49% yield.

8-[trans-3-(tert-Butyloxycarbonyl)vinyl]-1,3,7-trimethylxanthine (39). A mixture of 8-bromoxanthine (480 mg, 1.65 mmol), tert-butyl acrylate (0.380 mL, 2.60 mmol), Pd(AsO_3) $_2$ (8.7 mg, 19.5 μ mol), tri-*o*-tolylphosphine (20 mg, 65 μ mol), triethylamine (2 mL), and acetonitrile (2 mL) was warmed to 100 °C for 16 h with stirring in a capped tube. After cooling to room temperature, $CHCl_3$ was added and the mixture filtered. The organic layer was extracted twice with 1 N HCl, washed with brine several times, dried ($MgSO_4$), and then evaporated to dryness. The residue was treated with MeOH (1 mL), and hexane was added, to afford 182 mg of the crystalline product. The mother liquors were evaporated, and the remaining product was purified by preparative TLC (hexane/ethyl acetate 1:1) to give 49 mg (38% overall). Mp: 214–215 °C. 1H NMR (DMSO- d_6): δ 1.48 (s, 9H, CH_3), 3.23 (s, 3H, NCH_3), 3.43 (s, 3H, NCH_3), 4.03 (s, 3H, NCH_3), 6.73 (d, 1H, $J = 15$ Hz), 7.51 (d, 1H, $J = 15$ Hz). MS (CI NH_3): m/e 391 (MH $^+$).

6-Amino-8-[[4-(trifluoromethyl)benzoyl]amino]-1,3-di-methyluracil (40). 5,6-Diamino-1,3-dimethyluracil (2.06 g, 10 mmol) was suspended in 20 mL of dry pyridine and then 4-(trifluoromethyl)benzoyl chloride (2 mL, 14 mmol) was added and stirred for 4 h at room temperature. The mixture was then poured into 100 mL of water and the precipitate collected and recrystallized from $H_2O/EtOH$ (7:3) to give 1.6 g (55%) of colorless crystals. Mp: 287–289 °C. UV (MeOH): 225 (4.23), 287 (4.29).

6-Amino-8-[[4-(trifluoromethyl)benzoyl]amino]-1,3-di-*n*-propyluracil Monohydrate (41). In 10 mL of dry pyridine was dissolved 5,6-diamino-1,3-di-*n*-propyluracil (2.36 g, 10 mmol) and then after cooling 4-(trifluoromethyl)benzoyl chloride (2 mL, 11.8 mmol) was added slowly with stirring. The mixture was stirred at room temperature overnight and then evaporated and the residue treated with 100 mL of water with stirring until a precipitate was formed. The solid was collected and recrystallized from $H_2O/EtOH$ 7:3 to give 3.63 g (90%) of colorless crystals. Mp: 121–123 °C. UV (MeOH): 202 (4.29), 221 (4.30), 288 (4.27).

6-Amino-8-(4-(trifluoromethyl)benzoyl)-*N*-(methylamino)-1-methyluracil (42). 6-Amino-1-methyl-5-(methylamino)-uracil (2.6 g, 0.06 mol) was suspended in 100 mL of dry pyridine, and the mixture was cooled in an ice bath. 4-(Trifluoromethyl)benzoyl chloride (12 g, 58 mmol) added dropwise with stirring. After stirring for 4 h at room temperature, the mixture was evaporated and the residue treated with 100 mL of water to form a crystalline precipitate. Recrystallization from $H_2O/MeOH$ gave 12.45 g (71%) of colorless crystals. Mp: 288–300 °C. UV (MeOH): 205 (4.23), 214 sh (4.15), 287 (4.17).

6-Amino-8-benzoyl-*N*-(methylamino)-1-methyluracil (43). A suspension of 6-amino-1-methyl-5-(methylamino)uracil (17 g,

0.1 mol) in 200 mL of dry pyridine was cooled with ice and then benzoyl chloride (18 mL, 0.115 mol) was added dropwise with stirring. The reaction was continued overnight and then evaporated to dryness, and the residue was treated with 300 mL of H_2O to form a crystalline precipitate. Recrystallization from $H_2O/ethanol$ gave 21 g (76%) of colorless crystals. Mp: >300 °C. UV (MeOH): 203 (4.24), 232 sh (4.05), 288 (4.12).

6-Amino-8-(4-sulfonamidobenzoyl)-*N*-(methylamino)-1-methyluracil Monohydrate (44). A suspension of 6-amino-5-(methylamino)-1-methyluracil (17 g, 0.1 mol) in 600 mL of $H_2O/EtOH$ 1:1 was treated with 4-sulfamylbenzoic acid (30.1 g, 0.10 mol) and 1,3-dicyclohexylcarbodiimide (28 g, 0.12 mol) at room temperature with stirring. The crystalline precipitate was filtered to yield 2.94 g (83%) of chromatographically pure material. Mp: >300 °C. UV (MeOH): 202 (4.25), 222 (4.22), 288 (4.15).

Biology: Inhibition of binding of 1-nM-[3H]-*N*-(*N*-phenylisopropyl)adenosine (NEN, Boston, MA) to A_1 -receptors in rat brain membranes in rat brain membranes or of 4 nM [3H]-CGS 21680 (NEN, Boston, MA) to A_2 -receptors in rat striatal membranes was measured as described.²² Nonspecific binding was defined for A_1 -receptors with 10 μ M 2-chloroadenosine and for A_2 -receptors with 20 μ M 2-chloroadenosine. The K_i values were calculated from IC_{50} values by the method of Cheng and Prusoff²³ with K_D values of 1 nM for [3H]-*N*-(*N*-phenylisopropyl)adenosine and 14 nM for [3H]-CGS 21680.

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Structure-Activity Relationships in a Series of 8-Substituted Xanthines as Bronchodilator and A₁-Adenosine Receptor Antagonists

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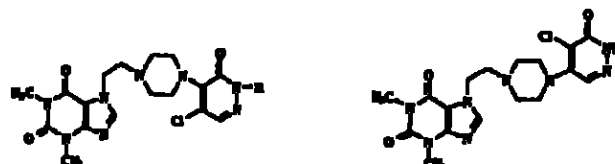
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Key Words: pyridazinones, bronchospasmolytic activity, A₁-adenosine affinity, structure-activity relationship

Summary

Four new derivatives of 8-piperazine-ethyl-xanthine were synthesized, and their bronchospasmolytic activity and A₁-adenosine affinity were studied. Their relaxant action in the tracheal muscle was lower than that of theophylline and that of theophylline derivatives substituted at the 7-position. Only compound 9, where the methyl group in the 1-position of the theophylline was substituted by an isobutyl group, shows a good affinity towards the A₁-adenosine receptor.

Furthermore, we also synthesized compound 9 in which the methyl group in the 1-position of theophylline was substituted by an isobutyl group, since substitution in this position is important for an affinity towards the A₁-adenosine receptor [6].



1a - R = H

1b - R = CH₃

2

Introduction

Theophylline is one of the most effective and frequently used drugs for the treatment of asthma. However, its adverse action on the cardiovascular and central nervous systems are well-known and impair its usefulness [1,2].

The bronchospasmolytic activity of theophylline can be attributed to an inhibition of phosphodiesterase and calcium mobilizers and an antagonist activity on the A₁-adenosine receptor. It is well known that adenosine has a significant role in the control of the central nervous, cardiovascular, and endocrine systems. The receptor adenosine has been subdivided into subtype A₁ and A₂, of which A₁ mediates inhibition of adenylate cyclase and is responsible for bronchoconstriction, whereas A₂ mediates stimulation of adenylate cyclase and is therefore responsible for bronchodilation.

A third subclass of adenosine receptors, A₃, has been claimed in the heart, and in the lung of the sheep and an interaction with this receptor leads to inhibition of adenylate cyclase [3].

Some pyridazinone derivatives show bronchospasmolytic activity since a high level of c-AMP can be maintained by an inhibition of phosphodiesterase [4].

We have recently reported some theophylline derivatives with several groups linked to the 7-position, such as 1a-b and 2, which show good broncholytic activity [5].

We therefore considered it to be of interest to extend our research through the introduction of the same piperazine-pyridazinone system into the 8-position of the xanthine nucleus (compounds 7, 8, 10) in order to obtain more information on structure-activity relationships and with the aim of designing new potent theophylline analogues with a larger margin of safety and a long duration of action.

Chemistry

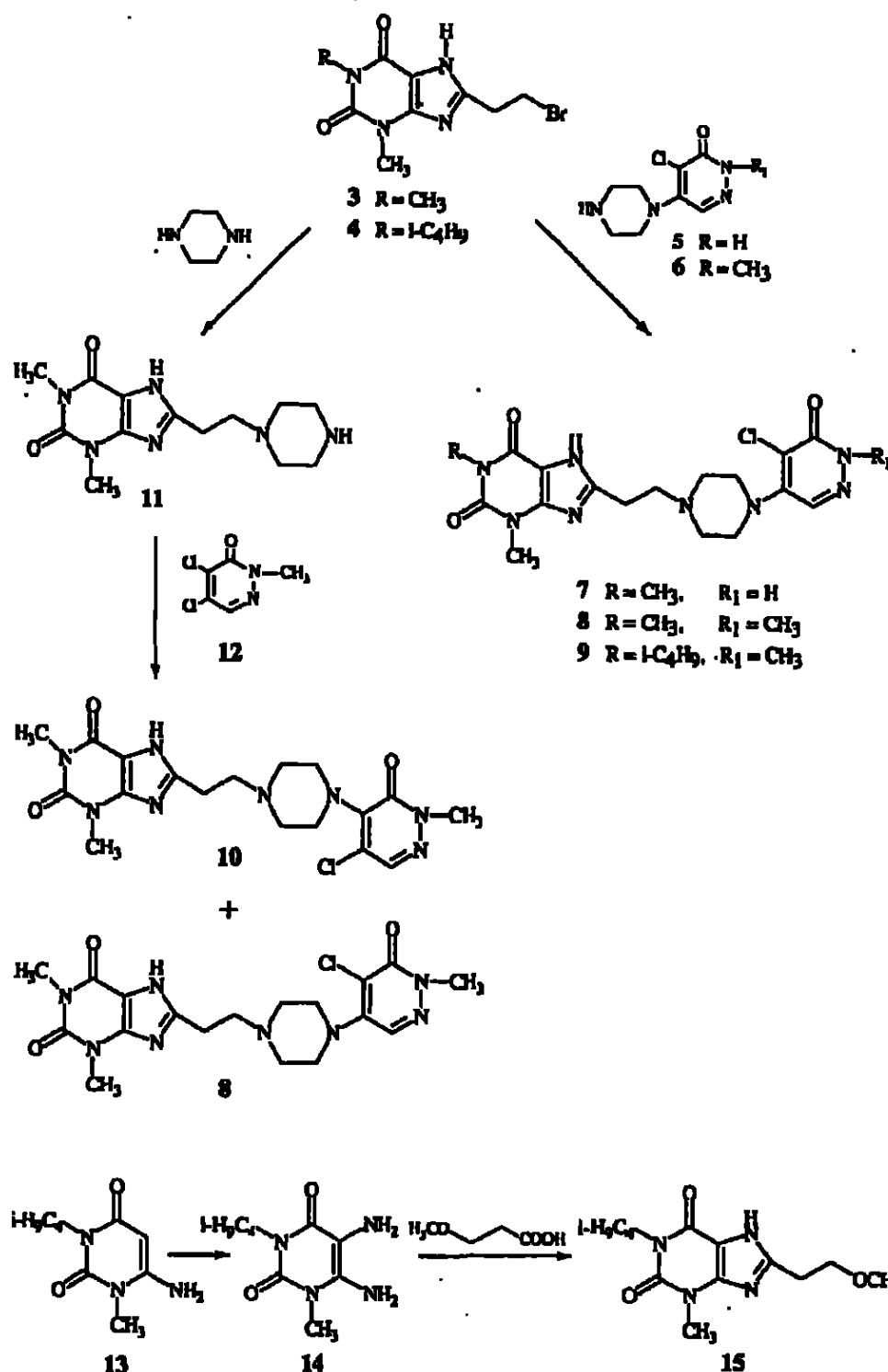
Alkylation of 1,3-dimethyl-8-(2-bromoethyl)xanthine (3) or 1-isobutyl-3-methyl-8-(2-bromoethyl)xanthine (4) with 4-chloro-5-(1-piperazinyl)-3(2H)-pyridazinone (5) or 2-methyl-4-chloro-5-(1-piperazinyl)-3(2H)-pyridazinone (6) in isoamyl alcohol and anhydrous K₂CO₃, gave compounds 7, 8, and 9. The 4- and 5-substituted isomers (compounds 8 and 10) were obtained by alkylation of compound 3 with piperazine in anhydrous EtOH, followed by substitution with 2-methyl-4,5-dichloro-3(2H)-pyridazinone (12) in 1,4-dioxane and K₂CO₃.

For the synthesis of 1-isobutyl-3-methyl-8-(2-bromoethyl)xanthine (4) compound 13 was used as starting material followed by reactions using to the methods of Traube [7] and Karlsson [8] (Scheme 1).

Results and Discussion

The broncholytic profile of these compounds was determined in vitro on guinea pig trachea and compared with aminophylline and theophylline using the method of Luduena [12].

The increased % in the velocity of the flux of perfusion was measured for these compounds. An increase in the perfusion flow rate of more than 50% indicated significant activity (Table 1). The biological results obtained for these compounds, in which the 1-ethyl-4-[3(2H)-pyridazinonyl]-piperazine system was linked in the 8-position of the xanthine nucleus, show that only compounds 8 and 9 have a low activity using a concentration of 100 µM. These results indicate that the introduction of the piperazine-pyridazinone

182
184

Scheme 1

system into the 8-position of the xanthine nucleus results in a decrease in the bronchospasmolytic activity compared with that of theophylline and of compounds 1a-b, 2^[5] in which the same systems are linked into 7-position.

Since xanthines act as inhibitors of the enzyme cyclic nucleotide phosphodiesterase and as A₁-adenosine receptor antagonists, for all compounds the affinity towards the A₁-adenosine receptor was determined and compared with that

of the compounds 1a-b and 2^[5]. These biological results show that although compounds 1a-b and 2 have bronchospasmolytic activity, they have not affinity towards the A₁-adenosine receptor. While compound 9, which shows a low bronchodilator activity, has on the other hand, a high affinity towards the A₁-adenosine receptor (Fig. 1) and this affinity is higher in comparison with that of theophylline, as demonstrated by Table 2.

Table 1. Evaluation of tracheal relaxation (*in vitro*)^{a)}.

Compound ^{b)}	Dose ($\mu\text{g/ml}$)	Tissue: guinea pig
		Increase % in the velocity of the flux of perfusion ^{c)}
Aminophylline	30	56.7 $\pm$ 5.7
Theophylline	30	61.0 $\pm$ 4.5
7	100	16.3 $\pm$ 3.3
	30	7.3 $\pm$ 3.5
8	100	58.7 $\pm$ 4.9
	30	43.0 $\pm$ 2.9
	10	24.3 $\pm$ 2.6
9	100	60.0 $\pm$ 2.6
	30	24.3 $\pm$ 1.8
10	100	45.0 $\pm$ 2.7
	30	32.0 $\pm$ 5.9
	10	13.3 $\pm$ 2.4
Adrenaline	0.25	100.0 $\pm$ 0.0

^{a)} The reported values are the average of three measurements.

^{b)} Partially soluble *in vitro*.

^{c)} The broncholytic data are the results of the screening performed by Pualabo Incorporated, Taiwan.

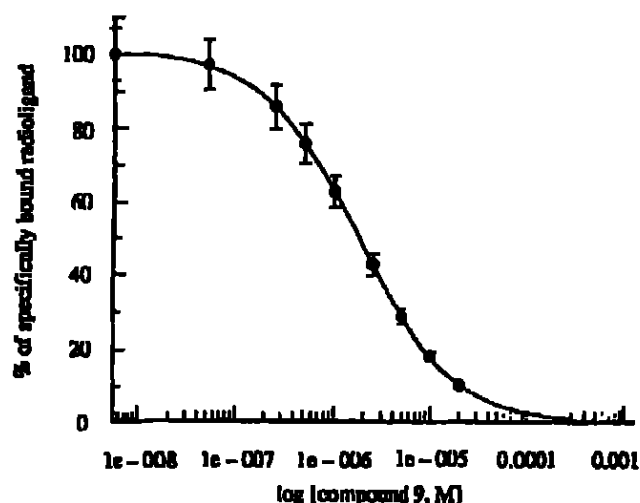


Figure 1. Modulation of [³H]-CHA binding by increasing concentrations of compound 9. Each point represents the mean $\pm$ SE of 4 independent determinations. The experiment was repeated 3 times with similar results.

We can therefore assume that bronchospasmodic activity is not necessarily connected with the affinity towards the but A₁-adenosine receptor.

Experimental Part

Mp: Rotchert microstage device, uncorr.—¹H-NMR spectra: Varian EM 390; 90 MHz and Bruker 200 MHz. CDCl₃, [D₆]DMSO, TMS int. stand.—Purity was checked by TLC.—Elemental analyses are within $\pm$ 0.4 % of theoretical values.

Table 2. Affinity for A₁-adenosine receptor^{a)}.

Compound	% Inhib. (10 μM)	K _i (μM) ^{b)}
Theophylline	23.0	10.6
1a	0.0	
1b	0.0	
2	0.0	
7	0.0	
8	48.0	
9	85.0	0.85
10	0.0	

^{a)} [³H]-CHA binding to bovine brain membranes. Inhibition percentages of specific binding are means of three determinations.

These compounds have been assayed also on the guinea pig cerebral cortex membranes. The biological results obtained are similar in comparison with that assayed on the bovine cerebral cortex membranes. This homology in the pharmacological results of the A₁-adenosine receptor is confirmed by Ueda et al.^[16]

^{b)} K_i values were calculated according to Cheng and Prusoff equation.

General method for the preparation of compounds 7, 8, and 9

1,3-Dimethyl-8-[2-(4-chloro-5-piperazin-3(2H)-pyridazinonyl)ethyl]-xanthine (7)

A mixture of 2.97 g (1×10^{-2} mole) of the bromo derivative 3^[9], 2.57 g (1.2×10^{-2} mole) of 4-chloro-5-(1-piperazinyl)-3(2H)-pyridazinone (5)^[10] and 1.6 g (1×10^{-2} mole) of K₂CO₃ in 80 ml of isomyllic alcohol was refluxed for 24 h. The mixture was filtered and the organic phase was evaporated under reduced pressure. The residue was purified by flash-chromatography using as eluent CH₂Cl₂/EtOH (7.5/2.5), yield: 30%, mp 288–292 °C.—¹H-NMR ([D₆]DMSO): δ (ppm) = 2.6–2.8 (m, 4H; H-pip.), 2.9–3.2 (m, 4H; 2CH₂), 3.3–3.5 (m, 7H; 4H-pip., N-CH₃), 3.6 (s, 3H; N-CH₃), 7.6 (s, 1H; 6-H-pyrid.), 11.5–9.5 (br s, 2H; NHCO, NH).—C₁₇H₂₁ClN₆O₃ (420.4) Calcd. C 48.5 H 5.0 N 26.6 Found C 49.0 H 5.3 N 26.8.

1,3-Dimethyl-8-[2-(2-methyl-4-chloro-5-piperazin-3(2H)-pyridazinonyl)ethyl]xanthine (8)

This compound was prepared in a similar way to compound 7 using as starting product 3 and 2-methyl-4-chloro-5-(1-piperazinyl)-3(2H)-pyridazinone (6)^[10], under reflux for 6 h, purified by flash-chromatography using as eluent CH₂Cl₂/EtOH (8/2) yield: 30%, mp 252–257 °C.—¹H-NMR (CDCl₃): δ (ppm) = 2.6–2.8 (m, 4H; H-pip.), 2.9–3.1 (m, 4H; 2CH₂), 3.3–3.5 (m, 7H; 4H-pip., N-CH₃), 3.6 (s, 3H; N-CH₃), 3.75 (s, 3H; N-CH₃), 7.55 (s, 1H; 6-H-pyrid.).—C₁₈H₂₃ClN₆O₃ (434.4) Calcd. C 49.7 H 5.3 N 25.8 Found C 50.0 H 5.0 N 25.3.

1-Isobutyl-3-methyl-8-[2-(2-methyl-4-chloro-5-piperazin-3(2H)-pyridazinonyl)ethyl]xanthine (9)

Was prepared by alkylation of the bromo derivative 4 and 6 using the same procedure described above; purified by flash-chromatography using as eluent CH₂Cl₂/EtOH (8.5/1.5), yield: 30%, mp 200–205 °C.—¹H-NMR (CDCl₃): δ (ppm) = 0.9 (d, 6H; J = 7 Hz, 2CH₃), 2.0–2.3 (m, 1H; CH-CH₃), 2.6–2.8 (m, 4H; H-pip.), 2.9–3.1 (m, 4H; 2CH₂), 3.3–3.5 (m, 4H; 4H-pip.), 3.6 (s, 3H; N-CH₃), 3.75 (s, 3H; N-CH₃), 3.85 (d, 2H; J = 7 Hz, N-CH₃), 7.5 (s, 1H; 6-H-pyrid.).—C₂₁H₂₈ClN₆O₃ (476.4) Calcd. C 52.9 H 6.1 N 23.5 Found C 53.3 H 6.5 N 23.7.

1,3-Dimethyl-8-(2-piperazinethyl)xanthine (11)

A mixture of 2.87 g (1×10^{-2} mole) of 1,3-dimethyl-8-(2-bromoethyl)xanthine (3), 1.03 g (1.2×10^{-2} mole) of piperazine in 60 ml of anhydrous EtOH was refluxed for 24 h. After cooling, the mixture was evaporated under reduced pressure. The residue was purified by alumina Brockmann II using $\text{CH}_2\text{Cl}_2/\text{EtOH}$ (8.5/1.5) as eluent, yield: 30%. $^1\text{H-NMR}$ (CDCl_3): δ (ppm) = 2.45–2.65 (m, 4H; H-pip.), 2.8–3.1 (m, 9H; 2CH_2 , 4H-pip., NH), 3.4 (s, 3H; N- CH_3), 3.6 (s, 3H; N- CH_3), 11.5 (s, 1H; NH).

1,3-Dimethyl-8-[2-(2-methyl-4-piperazin-5-chloro-3(2H)-pyridazinonyl)ethyl]xanthine (10) and 1,3-Dimethyl-8-[2-(2-methyl-4-chloro-5-piperazin-3(2H)-pyridazinonyl)ethyl]xanthine (8)

A mixture of 1.28 g (4.4×10^{-3} mole) of 8-(2-piperazinethyl)theophylline (11) 0.8 g (4.4×10^{-3} mole) of 2-methyl-4,5-dichloro-3(2H)-pyridazinone (12) [10] and 0.45 g (1.14×10^{-3} mole) of K_2CO_3 in 20 ml of 1,4-dioxane was heated to 80 °C for 15 h. After filtration, the organic phase was concentrated under reduced pressure. The residue was purified by flash-chromatography using $\text{CH}_2\text{Cl}_2/\text{EtOH}$ as eluent.

The 4-substituted isomer 10 was eluted with $\text{CH}_2\text{Cl}_2/\text{EtOH}$ (8.8/1.2), yield: 30%, mp 210–214 °C. $^1\text{H-NMR}$ (CDCl_3): δ (ppm) = 2.6–2.75 (m, 4H; H-pip.), 2.8–3.1 (m, 4H; 2CH_2), 3.4 (s, 3H; N- CH_3), 3.5–3.65 (m, 7H; 4H-pip., N- CH_3), 3.7 (s, 3H; N- CH_3), 3.85 (d, 2H; CH_2), 7.5 (s, 1H; 6-H-pyrid.), 12.0 (s, 1H; NH). $\text{C}_{21}\text{H}_{25}\text{ClN}_5\text{O}_3$ (434.4) Calcd. C 49.7 H 5.3 N 25.8 Found C 50.1 H 5.5 N 25.4.

The 5-substituted isomer 8 was eluted with $\text{CH}_2\text{Cl}_2/\text{EtOH}$ (8.5/1.5), yield: 15%, mp 253–257 °C. For spectral data see above.

1-Isobutyl-3-methyl-4-aminouracil (13)

A mixture of 3.04 g (2.4×10^{-2} mole) of 3-methyl-4-aminouracil [11], 7 ml of NaOH 15% and 15 ml of EtOH 95% was refluxed for 30 min. This solution was poured into an autoclave, 8.7 g (4.8×10^{-2} mole) of the isobutyl iodide was added and the mixture was heated at 100 °C for 4 h. At the end of the heating period, the alcohol and excess alkyl iodide were evaporated *in vacuo*. The residue was digested with hot EtOH and filtered. The organic phase was evaporated *in vacuo* and the residue purified on alumina Brockmann II using $\text{CH}_2\text{Cl}_2/\text{EtOH}$ (9/1) as eluent, yield: 35%, mp 150–154 °C. $^1\text{H-NMR}$ (CDCl_3): δ (ppm) = 0.95 (d, 6H; $J = 7\text{Hz}$, 2CH_3), 2.0–2.2 (m, 1H; CH-CH_3), 3.35 (s, 3H; N- CH_3), 3.7 (d, 2H; $J = 7\text{Hz}$, N- CH_2), 5.0 (br s, 1H; CH), 5.4 (br s, 2H; NH₂).

1-Isobutyl-3-methyl-4,5-diaminouracil (14)

0.21 g of NaNO_2 was added to a cooled (5 °C) stirred mixture containing 0.65 g (3.3×10^{-3} mole) of compound 13 in water and this was acidified with acetic acid. A deep-purple color characteristic of the nitroso derivative appeared immediately, and stirring was continued at room temperature for 20 h. The mixture was filtered and the crude product was treated with 0.5 ml of $(\text{NH}_4)_2\text{S}$. The mixture was stirred at room temperature for 2 h, and evaporated. The residue was treated with EtOH and the solid was filtered off, thoroughly washed with EtOH and dried at room temperature *in vacuo* and the crude product was used without further purification. Yield: 70%, mp 160–170 °C. $^1\text{H-NMR}$ ($\text{D}_2\text{O}/\text{DMSO}$): δ (ppm) = 0.9 (d, 6H; $J = 7\text{Hz}$, 2CH_3), 1.9–2.2 (m, 1H; CH-CH_3), 2.5 (br s, 2H; NH₂), 3.3 (s, 3H; N- CH_3), 3.6 (d, 2H; $J = 7\text{Hz}$, N- CH_2), 6.0 (br s, 2H; NH₂).

1-Isobutyl-3-methyl-8-(2-methoxyethyl)xanthine (15)

A mixture of 1.9 g (1×10^{-2} mole) of 14 and 4 g of 3-methoxypropionic acid was stirred and heated at 100 °C from 4 h. After cooling to 20 °C the solution was stirred for 20 h and treated with Et₂O (50 ml). The mixture was filtered and the crude product (yield 50%) was treated with 4 ml of water and 1 ml of NaOH 12N and heated at 100 °C for 2 h; after cooling at 5 °C the mixture was made acidic (pH 6) by the addition of H₂SO₄ 4N and the precipitate was filtered off and crystallized with EtOH, yield: 40%, mp 175–182 °C. $^1\text{H-NMR}$ (CDCl_3): δ (ppm) = 0.9 (d, 6H; $J = 7\text{Hz}$, 2CH_3), 2.1–2.3 (m, 1H; CH-CH_3), 3.1 (d, 2H; $J = 7\text{Hz}$, CH_2), 3.4 (s, 3H; N- CH_3), 3.6 (s, 3H; OCH₃), 3.75–3.95 (m, 4H; 2CH_2).

1-Isobutyl-3-methyl-8-(2-bromoethyl)xanthine (4)

A solution of 1.5 g (5.3×10^{-3} mole) of 15 in 40 ml of 48% HBr, was stirred and heated under reflux for 8 h, with slow bubbling of dry HBr into it solution. After completion, the solution was evaporated *in vacuo* and the residue crystallized with H₂O, yield: 50%, mp 170–177 °C. $^1\text{H-NMR}$ (CDCl_3): δ (ppm) = 1.0 (d, 6H; $J = 7\text{Hz}$, 2CH_3), 2.0–2.3 (m, 1H; CH-CH_3), 3.45 (d, 2H; $J = 7\text{Hz}$, CH_2), 3.6 (s, 3H; N- CH_3), 3.7–3.9 (m, 4H, 2CH_2), 12.5 (s, 1H; NH).

Pharmacology**Material and Methods****Bronchial relaxation (in vitro)**

The isolated guinea pig lung was perfused and constricted with physiological salt solution containing adrenaline (0.05 µg/ml) at 37 °C. The substance (µg/ml) was injected as a bolus just proximal to the entry of the cannula into the trachea. An increase in the perfusion flow rate of more than 50% (>0) indicated significant activity. The increased % in the velocity of the flux of perfusion was calculated using the method of Luduen [13].

A₁-Adenosine receptor binding assay

Compounds were assayed for their ability to inhibit the binding of the selective A₁ agonist radioligand ^3H -CHA to bovine cerebral cortex membranes as previously described [13].

After homogenization in 0.32 M sucrose and protease inhibitors, the homogenate was centrifuged at 1000 × g for 10 min at 4 °C and the pellet discarded. The supernatant was recentrifuged at 48000 × g for 15 min at 4 °C. The resulting pellet was resuspended in 10 volumes of 50 mM Tris-HCl, pH 7.7, with protease inhibitors and 2 mM MgCl₂, homogenized, and centrifuged at 48000 × g for 15 min at 4 °C. The final pellet was resuspended in 10 volumes of fresh buffer, incubated with adenosine deaminase (2 U/ml) at 37 °C for 60 min in order to remove endogenous adenosine then recentrifuged at 48000 × g for 15 min at 4 °C. The pellet was the resuspended in incubation buffer and used in the binding assays. The ^3H -CHA binding assay was performed in triplicate by incubating aliquots of the membrane fraction (0.2–0.3 mg protein) with 1.2 nM ^3H -CHA (New England Nuclear 34.4 Ci/mM) and the test compound in a final volume of 0.5 ml of Tris-HCl buffer, at 25 °C for 45 min. Non-specific binding was defined as the presence of 10 µM (*R*)-*N*-(6)-(phenylisopropyl)adenosine (*R*-PIA). The reaction was terminated by the addition of 5 ml of ice-cold incubation buffer filtered through Whatman GF/B glass fiber filters under suction and washed with 2 × 5 ml ice cold Tris-buffer.

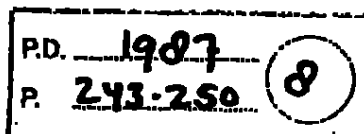
Radioactivity was counted in 10 ml of Ready Protein Beckman scintillation cocktail in a LS1800 scintillation counter. Specific binding was obtained by subtracting non-specific binding from total binding and approximated 75–85% of total binding. Unless otherwise stated, all tested compounds were dissolved in pure DMSO and added to the incubation mixture. Blank experiments were carried out to determine the effect of the solvent on binding. DMSO (2%) was found to decrease control binding by 5–10%. Prote estimation was based on a reported method [14] after membrane solubilization with 0.75 N NaOH, using bovine serum albumin as standard. The concentration of the tested compounds that produces 50% inhibition of the specific ^3H -CHA binding (IC_{50}) was determined by log-probit analysis with several concentrations of the displacers, each performed in triplicate. The K_i values were calculated from IC_{50} values using the equation of Cheng and Prusoff [15], $K_i = \text{IC}_{50}/(1 + I/K_d)$.

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Original paper

New xanthine derivatives with potent and long lasting anti-bronchoconstrictive activity

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Summary — Twenty eight new derivatives of 8-aminoalkyl substituted xanthine have been synthesized. They all have demonstrated a potent anti-bronchoconstrictive effect in the guinea pig and some of them have a very long duration of action (> 48 h). Among them, one compound: 1-methyl 3-isobutyl 8-(4-benzhydryl piperazino ethyl) xanthine (57) (S 9795) has been selected for clinical trials in asthmatic patients because of its long duration of action, its lack of CNS stimulating effects and its inhibiting action on mast cell degranulation and phosphodiesterase activity.

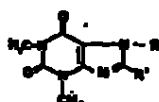
Résumé — Nouveaux dérivés de xanthines à activité anti-bronchoconstrictrice puissante et de longue durée. Vingt-huit nouveaux dérivés d'aminoalkyl-8 xanthines ont été synthétisés. Tous montrant un puissant effet anti-bronchoconstricteur chez le cobaye et quelques-uns ont une très longue durée d'action (> 48 h). Parmi ceux-ci le composé: méthyl-1 isobutyl-3 ((benzhydryl-4 piperazino)ethyl)-8 xanthine (57) (S 9795) qui présente une absence d'effets stimulants du SNC, une action inhibitrice puissante de la dégranulation mastocytaire et de la phosphodiesterase a été sélectionné en vue d'études cliniques chez des patients asthmatiques.

8-aminoalkyl substituted xanthines / benzhydryl piperazines / piperidines / ethylmedazoles / anti-bronchoconstrictors / phosphodiesterase inhibitors / asthma

Introduction

For quite some time, theophylline I has been recognized as a potent bronchodilator for the relief of acute asthma attacks. Unfortunately, its use was made difficult because of its many side effects which give it a very low margin of safety.

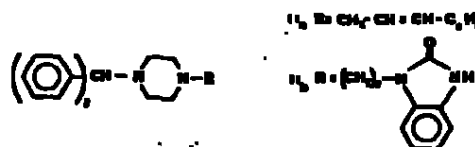
More recently, the application of new knowledge related to the pharmacodynamic and pharmacokinetic characteristics of this drug (poor water solubility and bad absorption from the gut) led to better control of blood levels and consequently improved both its efficacy and safety in asthma treatment. To increase its water solubility, a number of N-7 substituted methyl xanthines had been synthesized (dyphylline Ia, etophylline Ib, proxiphylline Ic, bamifylline Id).



- I R' = H R = H
Ia R' = H R = CH₂CHOHCH₂OH
Ib R' = H R = CH₂CH₂OH
Ic R' = H R = CH₂CHOHCH₃
Id R' = CH₂C₄H₉ R = (CH₂)₃N(C₂H₅) (CH₂CH₂OH)

These derivatives are stable in solution or *in vivo* but they are more closely related to the caffeine than the theophylline structure. Moreover, they are generally less active and present the same disadvantages as theophylline for both the duration of action and CNS stimulating properties.

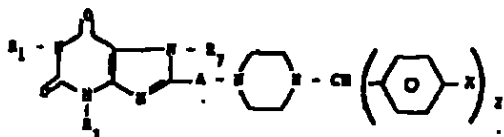
With the aim of designing new more potent theophylline analogues, useful in the treatment of asthma with a larger margin of safety and a long duration of action, we became interested in the compounds inhibiting both the release and the effects of allergic mediators such as cinnarizine IIa [1] and oxatamide IIb [2]. These two compounds contain



a diphenyl methyl piperazino group in their molecules which confer upon them anti-allergic—anti-histaminic properties. Furthermore, in the methyl xanthine series, the alkyl substitution at the 8-position made the compounds more active than theophylline, on tracheal relaxation in

184
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Table I. 8-[4-(Substituted benzhydryl)piperazino alky]-1,3,7-substituted xanthines.



N ^o	R ₁	R ₂	R ₃	A	X	yield %	mp base or salt °C (b)	Crystn solvent (a)	FORMULA (c)	Oral toxicity in mice mg/kg po	Histamine aerosol ED 50 mg/kg po (h)	PDE IC 50 M (i)
53	CH ₃	CH ₃	H	CH ₂	H	46	241 (k)	MeOH	C ₂₉ H ₃₂ N ₆ O ₂	> 1600	20 (k)	NT
54	CH ₃	CH ₃	H	(CH ₂) ₂	H	22	103-106 (cap)	CH ₂ Cl ₂	C ₃₀ H ₃₄ N ₆ O ₂	> 1600	10 > 20	NT
55	CH ₃	CH ₃	H	(CH ₂) ₃	H	42	249-250 (cap)	MeOH	C ₃₁ H ₃₆ N ₆ O ₂ ·HCl	> 1600	10 > 20	NT
56	CH ₃	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₃	H	34	217-220 (cap)	EtOH	C ₃₀ H ₃₄ N ₆ O ₂ ·2HCl	> 1600	0.7 10	4.1·10 ⁻⁴
57	CH ₃	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂	H	72	202 (k)	AcOEt	C ₂₉ H ₃₂ N ₆ O ₂	> 3200	0.6 6	6.10 ⁻⁴
58	CH ₃	CH ₃	CH ₃	(CH ₂) ₃	H	52	198 (k)	EtOH	C ₃₂ H ₃₆ N ₆ O ₂ ·C ₆ H ₄ O ₄ (d)	1500	1 > 20	8.2·10 ⁻³
59	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₃	H	30	182 (k)	EtOH Et ₂ O	C ₃₁ H ₃₄ N ₆ O ₂ ·C ₆ H ₄ O ₄ (d)	NT	0.7 10	4.9·10 ⁻³
60	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₂	H	70	214-218 (cap)	n-PrOH Et ₂ O	C ₃₀ H ₃₂ N ₆ O ₂ ·2HCl	> 1600	1 > 20	6.02·10 ⁻³
61	CH ₃	C ₆ H ₅	CH ₃	(CH ₂) ₃	H	59	150 (k)	isoPrOH	C ₃₂ H ₃₈ N ₆ O ₂	> 1600	2.5 > 20	3.74·10 ⁻³
62	CH ₃	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂	F	45	184 (k)	AcOEt	C ₃₀ H ₃₂ F ₂ N ₆ O ₂	> 1600	2.5 10	9.7·10 ⁻⁴
63	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₂	F	59	174 (k)	n-PrOH	C ₃₀ H ₃₂ F ₂ N ₆ O ₂ ·2C ₆ H ₄ O ₄ (e)	> 1600	2.5 > 20	1.6·10 ⁻⁴
64	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₃	F	55	180 (k)	EtOH	C ₃₁ H ₃₄ F ₂ N ₆ O ₂ ·C ₆ H ₄ O ₄ (d)	1600	2.5 20	1.8·10 ⁻⁴
65	CH ₃	n-C ₃ H ₇	CH ₃	(CH ₂) ₃	H	65	200 (k)	EtOH	C ₃₀ H ₃₄ N ₆ O ₂ ·C ₆ H ₄ O ₄ (d)	800	1 > 20	5.25·10 ⁻³
66	C ₂ H ₅	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₃	H	19	201-205 (cap)	n-PrOH	C ₃₁ H ₃₆ N ₆ O ₂ ·C ₆ H ₄ O ₄ (d)	1600	1 20	4.10 ⁻³
67	C ₂ H ₅	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₃	H	61	193 (k)	n-PrOH	C ₃₂ H ₄₀ N ₆ O ₂ ·C ₆ H ₄ O ₄ (e)	> 800	2.5 > 20	> 10 ⁻⁴
68	CH ₃	CH ₂ CH(CH ₃) ₂	H	CH ₂	H	37	182 (k)	MEC	C ₂₈ H ₃₀ N ₆ O ₂	> 800	5 > 20	NT
69	n-H	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂	H	30	240 (k)	AcOEt	C ₂₉ H ₃₂ N ₆ O ₂	> 3200	1 5	1.10 ⁻³
70	H	CH ₂ C ₆ H ₅	H	(CH ₂) ₂	H	50	232-235 (cap)	AcOEt	C ₃₁ H ₃₄ N ₆ O ₂	> 3200	5 > 20	NT
71	CH ₃	CH ₂ CH(CH ₃) ₂	(f)	(CH ₂) ₂	H	33	145-155 (cap)	isoPrOH Et ₂ O	C ₃₂ H ₄₀ N ₆ O ₄ ·2HCl	3200	5 > 20	NT
72	CH ₃	CH ₂ CH(CH ₃) ₂	(g)	(CH ₂) ₂	H	71	190-200 (cap)	isoPrOH Et ₂ O	C ₃₁ H ₃₆ N ₆ O ₄ ·2HCl	1600	10 > 20	NT
73	CH ₃	CH ₂ CH(CH ₃) ₂	H	-CHOH-CH ₂	H	17	212 (k)	Et ₂ O	C ₂₉ H ₃₂ N ₆ O ₃	> 800	10 > 20	NT
74	H	CH ₂ CH(CH ₃) ₂	H	-CHOH-CH ₂	H	21	234 (k)	AcOEt	C ₂₈ H ₃₀ N ₆ O ₃	> 1600	7.5 > 20	NT
									Aminophylline	800	49 (k)	1.2·10 ⁻⁴
									Mepyramine	NT	0.4 (k)	10 ⁻⁴ (k)

(a) MeOH: methanol; EtOH: ethanol; PrOH: propanol; Et₂O: ethyl ether; AcOEt: ethyl acetate; MEC: methyl ethyl ketone.

(b) and (c) See corresponding footnotes for Table VII.

(d) As fumarate salt.

(e) As maleate salt.

(f) R₃ is a 2,3-dihydroxy propyl group.(g) R₃ is a 2-hydroxyethyl group.

(h) 45 min after oral administration.

(i) 48 h after oral administration.

(j) Phosphodiesterase inhibition (studied on rat brain tissue).

(k) Inactive.

guinea pigs [3, 4]. Hence, it became interesting to synthesize new xanthine compounds substituted at the 8-position with a benzhydryl piperazino alkyl group, with the structure III:

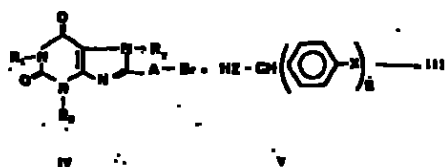


Each parameter R_1 , R_2 , R_3 , A and X was varied selectively: R_1 can be a hydrogen atom or low alkyl radicals; R_2 = low alkyl radicals, benzyl or phenyl groups; A = a $(CH_2)_m$ unbranched chain optionally substituted with an OH radical; X = a hydrogen or fluor atom; and R_3 a hydrogen atom, lower alkyl or hydroxy alkyl radicals (Table I).

We also studied the influence of the substitution of the piperazine nucleus by N, N' -diethylethylenediamine, piperidine or oxypiperidine moieties (Table II) and the variations of the distance (m 1—3) between these nuclei and the xanthine nucleus.

Chemistry

The syntheses of these compounds III (Table I) were performed according to the general method (Scheme 1):

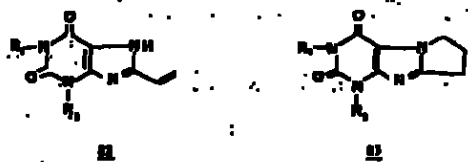


Scheme 1

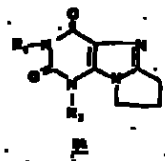
where R_1 = H or low alkyl radicals; Z = piperazino, piperidino or N, N' -diethylethylenediamino groups.

A substituted 8-bromo alkyl or bromo hydroxy alkyl xanthine IV was condensed with an appropriate substituted-2-moiety V, such as 4-(substituted benzhydryl) piperazine, 4-(fluoro benzhydryl) piperidine, 4-benzhydryloxy piperidine or N -benzhydryl- N, N' -diethylethylenediamine, in alcohol.

During the above mentioned reaction, a small quantity of each of the three by-products of general structures 82, 83 and 84 has been chromatographically isolated when A was $(CH_2)_{2,3}$ (Table II). The isomers 83 and 84 a and b

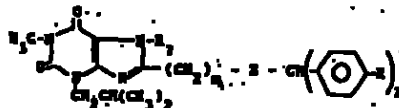


- $R_1 = CH_3$
- $R_2 = CH_3$
- $R_3 = CH_2CH(CH_3)_2$
- $R_1 = CH_3$
- $R_2 = CH_3$
- $R_3 = CH_2CH(CH_3)_2$



- $R_1 = CH_3$
- $R_2 = CH_3$
- $R_3 = CH_2CH(CH_3)_2$

Table II. 1-Methyl-3-isobutyl-7-alkyl-8-substituted xanthines.



N	R ₁	R ₂	Z	X	Yield %	cryst. solvent	mp base or salt °C (lit)	FORMULA	anal. purity by mass, mp, %	Molecular weight		P ₅₀ (log)
										(a)	(b)	
21	H	2	H ₂ C=N-(CH ₂) ₂ -N-C ₆ H ₅	H	15	isoPrOH Et ₂ O	135-140 (exp)	C ₂₁ H ₂₈ N ₆ O ₄ 2HCl	900	10	0	1.5-1
26	CH ₃	2	H ₂ C=N-(CH ₂) ₂ -N-C ₆ H ₅	H	48	isoPrOH Et ₂ O	130-140 (exp)	C ₂₂ H ₃₀ N ₆ O ₄ 2HCl	900	20	0	5.10-1
27	CH ₃	3	H ₂ C=N-(CH ₂) ₃ -N-C ₆ H ₅	H	49	isoPrOH Et ₂ O	135-138 (exp)	C ₂₃ H ₃₂ N ₆ O ₄ 2HCl	> 900	> 10	> 20	5.8.10-1
28	H	2		H	37	isoPrOH Et ₂ O	163-167 (exp)	C ₂₁ H ₂₈ N ₆ O ₄ 2HCl	1000	5	10	2.6.10-1
29	CH ₃	2		H	80	n-PrOH	173-177 (exp)	C ₂₂ H ₃₀ N ₆ O ₄ 2HCl	1600	2.5	> 20	1.2.10-1
30	CH ₃	3		H	77	EtOH	194 (a)	C ₂₃ H ₃₂ N ₆ O ₄ 2HCl	900	2.5	10	5.5.10-1
31	H	2		H	72	AcOH	200 (a)	C ₂₁ H ₂₆ N ₄ O ₄	> 900	2.5		5.2.10-1

(a), (b), (c), (d), (h), (i), (j), (k) See corresponding footnotes for Table III.

Table III

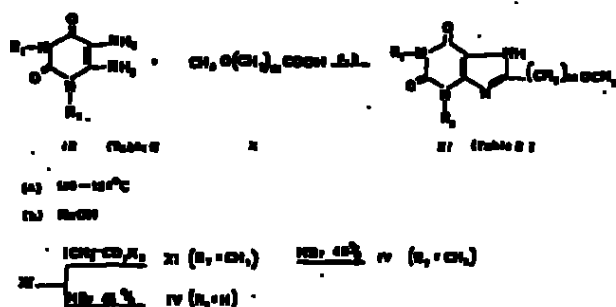
R ¹	R ₂	mp base °C (lit)	FORMULA (d)	¹ H NMR δ (ppm)
IIa	CH ₃	215 (cap)	C ₉ H ₁₀ N ₄ O ₂	(DMF-d ₇): 6.5-7.7 (m, 3H, CH = CH ₂); 1.4 (s, 3H, N-CH ₃); 3.25 (s, 3H, O = C ₂ N-CH ₃)
IIb	CH ₃	223 (d)	C ₁₂ H ₁₆ N ₄ O ₂	(DMF-d ₇): 6.5-7.8 (m, 3H, CH = CH ₂); 1.5 (s, 3H, N-CH ₃); 1.3 (s, 3H, N-CH ₃); 1.3 (s, 3H, N-CH ₃); 2.3 (m, 1H, CH (CH ₃) ₂); 0.9 (d, 6H, CH (CH ₃) ₂)
IIc	CH ₃	206 (d)	C ₁₀ H ₁₂ N ₄ O ₂	(CDCl ₃): 4.3 (t, 2H, N-CH ₂); 3.5 (s, 3H, O = C ₂ N-CH ₃); 1.4 (s, 3H, N-CH ₃); 2.8 (m, 4H, CH ₂ -CH ₂)
IIId	CH ₃	148 (d)	C ₁₃ H ₁₈ N ₄ O ₂	(CDCl ₃): 4.3 (t, 2H, N-CH ₂); 3.5 (s, 3H, O = C ₂ N-CH ₃); 1.4 (s, 3H, N-CH ₃); 2.8 (m, 4H, CH ₂ -CH ₂); 2.1 (m, 1H, CH (CH ₃) ₂); 1.6 (d, 6H, CH (CH ₃) ₂)
IIe	C ₄ H ₉	194 (d)	C ₁₄ H ₂₀ N ₄ O ₂	(CDCl ₃): 4.3 (t, 2H, N-CH ₂); 4.1 (s, 2H, N-CH ₂ -CH ₃); 4.3 (s, 2H, N-CH ₂ -CH ₃); 2.9 (m, 4H, CH ₂ -CH ₂); 2.3 (m, 1H, CH (CH ₃) ₂); 1.3 (s, 3H, N-CH ₃); 1.6 (d, 6H, CH (CH ₃) ₂)
IIIf	CH ₃	235 (d)	C ₁₀ H ₁₂ N ₄ O ₂	(CDCl ₃): 4.4 (t, 2H, N-CH ₂); 1.6 (s, 3H, O = C ₂ N-CH ₃); 1.4 (s, 3H, N-CH ₃); 2.8 (m, 4H, CH ₂ -CH ₂)
IIIg	CH ₃	194 (d)	C ₁₃ H ₁₈ N ₄ O ₂	(CDCl ₃): 4.3 (t, 2H, N-CH ₂); 4.1 (s, 2H, N-CH ₂ -CH ₃); 1.4 (s, 3H, N-CH ₃); 2.9 (m, 4H, CH ₂ -CH ₂); 2.3 (m, 1H, CH (CH ₃) ₂); 1.6 (d, 6H, CH (CH ₃) ₂)

(b) (c) See corresponding footnotes for Table VII.

have closely related NMR, IR and mass spectra. However, the UV spectrum of compounds 83 is the same as that of caffeine (max 275 nm, ε 9500), whereas the UV spectrum of 84 shows two maxima (272 nm, ε 9750 and 237 nm, ε 8500).

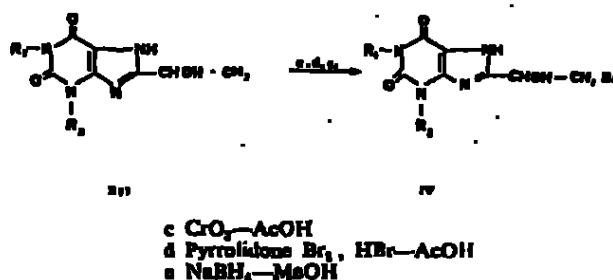
The compounds III (R₁ = H) can be hydroxyalkylated at the 7-position, in dimethylformamide (DMF), in the presence of potassium carbonate, by means of chloroethanol or 1-chloropropanediol (see Experimental protocols).

The starting material IV (Table IV) in which A = (CH₂)_n was prepared by analogy with a literature method [4] according to Scheme 2 (method A).



Scheme 2

When A = —CHOH—CH₂—, the starting material IV was prepared by analogy with a literature method [5] according to method B (Scheme 3).



Scheme 3

Results and Discussion

In a general manner, the substitution at the 8-position of the xanthine nucleus leads, in every case, regardless of the nature of the amino groups (piperazine or Z) (Tables I and II), to compounds (53—81) which are 2- to 80-fold more active than aminophylline (AP), in preventing histamine-induced bronchoconstriction in unanesthetized guinea pigs, whereas toxicities are of the same order or less important than that of AP. The replacement of the 3-methyl group by a 3-isobutyl group with the aim of avoiding a very fast metabolism by demethylation as in theophylline leads, in every case, regardless of the nature of R₁ or the amino group, to compounds with a very long (> 48 h) duration of action (56, 57, 59, 62, 69, 78, 80 and 81).

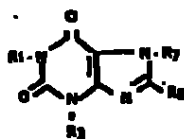
The anti-histaminic reference drug, mepyramine, shares the protective activity against histamine-induced bronchoconstriction, but this activity completely disappeared 48 h (even 24 h) after treatment (Table I).

The effectiveness of several compounds in inhibiting histamine granule release from rat peritoneal mast cells was determined (Table V). Compound 57 was found to be about 100-times more active than theophylline and about 200-times more active than cromoglycate. This difference may be related to the short acting character of this latter drug. On the other hand, as previously reported for several anti-histaminics [15], mepyramine potentiated the effect of 48/80. Then, the mode of action of compound 57 is clearly different from that of classical anti-histaminics.

As shown in Table VI, the spasmolytic activity of 57 against Ca-induced contractions of *taenia coli* is concentration dependent with an IC₅₀ value of $4.73 \pm 0.19 \times 10^{-6}$ M. The inhibitory activity of 57 is very similar to that of papaverine and flunarizine, but this property of 57 contrasts with the very low activity of its congeneric compounds aminophylline and enprofylline (IC₅₀ 1.4×10^{-3} M and 1.1×10^{-3} M, respectively).

The studied compounds are potent inhibitors of phosphodiesterase (PDE) (Tables I and II). Three compounds 56, 57 and 62, unsubstituted at the 7-position, have IC₅₀ of 10^{-6} M *in vitro* (Table I) and are from 12- to 30-fold more

Table IV. 1,3,7,8-Substituted xanthines.



No.	R ₁	R ₂	R ₃	R ₄	methods of prep.	yield, %	cryst solvent (a)	mp (°C) (b)	FORMULA (d)
18	CH ₃	CH ₃	H	(CH ₂) ₂ OCH ₃	A	47	CH ₂ Cl ₂	192	C ₁₆ H ₁₆ N ₄ O ₇
19	CH ₃	CH ₃	H	(CH ₂) ₂ OCH ₃	A	53	CH ₂ Cl ₂	192	C ₁₆ H ₁₆ N ₄ O ₇
20	CH ₃	CH ₃	CH ₃	(CH ₂) ₂ OCH ₃	A	73	isoPrOH	198	C ₁₇ H ₁₈ N ₄ O ₇
21	CH ₃	CH ₃	CH ₃	(CH ₂) ₂ OCH ₃	A	81	isoPrOH	99	C ₁₇ H ₁₈ N ₄ O ₇
22	CH ₃	CH ₂ CH(CH ₃) ₂	H	CH ₂ OCH ₃	A	68	H ₂ O	198	C ₁₇ H ₂₀ N ₄ O ₇
23	CH ₃	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ OCH ₃	A	69	H ₂ O	189	C ₁₇ H ₂₀ N ₄ O ₇
24	CH ₃	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ OCH ₃	A	92	H ₂ O	144	C ₁₆ H ₁₈ N ₄ O ₇
25	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₂ OCH ₃	A	88	cyclo H	83	C ₁₈ H ₂₂ N ₄ O ₇
26	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₂ OCH ₃	A	89	cyclo H	76	C ₁₈ H ₂₂ N ₄ O ₇
27	CH ₃	CH ₂ CH = CH ₂	H	(CH ₂) ₂ OCH ₃	A	71	H ₂ O	188	C ₁₇ H ₁₈ N ₄ O ₇
28	CH ₃	CH ₂ CH = CH ₂	CH ₃	(CH ₂) ₂ OCH ₃	A	100	CH ₂ Cl ₂	44-45	C ₁₈ H ₂₀ N ₄ O ₇
29	CH ₃	n-C ₃ H ₇	CH ₃	(CH ₂) ₂ OCH ₃	(d)	98	EtOH	67	C ₁₈ H ₂₂ N ₄ O ₇
30	C ₂ H ₅	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ OCH ₃	A	91	H ₂ O	137-139	C ₁₇ H ₂₀ N ₄ O ₇
31	C ₂ H ₅	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₂ OCH ₃	A	75	CH ₂ Cl ₂	80	C ₁₈ H ₂₂ N ₄ O ₇
32	CH ₃	C ₆ H ₅	H	(CH ₂) ₂ OCH ₃	A	51	H ₂ O	218	C ₁₈ H ₁₈ N ₄ O ₇
33	CH ₃	C ₆ H ₅	CH ₃	(CH ₂) ₂ OCH ₃	A	90	isoPrOH	163	C ₁₉ H ₂₀ N ₄ O ₇
34	H	CH ₂ CH ₂ CH ₃	H	(CH ₂) ₂ OCH ₃	A	86	H ₂ O	226	C ₁₉ H ₁₈ N ₄ O ₇
35	H	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ OCH ₃	A	55	H ₂ O	230	C ₁₇ H ₁₈ N ₄ O ₇
36	CH ₃	CH ₃	H	(CH ₂) ₂ Br	A	75	H ₂ O	227	C ₉ H ₁₁ BrN ₄ O ₂
37	CH ₃	CH ₃	H	(CH ₂) ₂ Br	A	86	H ₂ O	229	C ₁₀ H ₁₃ BrN ₄ O ₂
38	CH ₃	CH ₃	CH ₃	(CH ₂) ₂ Br	A	73	CH ₂ Cl ₂	130	C ₁₁ H ₁₅ BrN ₄ O ₂
39	CH ₃	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ Br	A	96	H ₂ O	224	C ₁₁ H ₁₅ BrN ₄ O ₂
40	CH ₃	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ Br	A	88	H ₂ O	210	C ₁₂ H ₁₇ BrN ₄ O ₂
41	CH ₃	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ Br	A	82	H ₂ O	161	C ₁₃ H ₁₉ BrN ₄ O ₂
42	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₂ Br	A	85	CHCl ₃	114	C ₁₃ H ₁₉ BrN ₄ O ₂
43	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₂ Br	A	67	CHCl ₃	108	C ₁₄ H ₂₁ BrN ₄ O ₂
44	CH ₃	n-C ₃ H ₇	CH ₃	(CH ₂) ₂ Br	A	76	CH ₂ Cl ₂	92	C ₁₅ H ₂₃ BrN ₄ O ₂
45	CH ₃	n-C ₃ H ₇	H	(CH ₂) ₂ Br	A	76	H ₂ O	168	C ₁₅ H ₂₁ BrN ₄ O ₂
46	CH ₃	C ₆ H ₅	CH ₃	(CH ₂) ₂ Br	A	77	H ₂ O	156	C ₁₅ H ₁₇ BrN ₄ O ₂
47	C ₂ H ₅	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ Br	A	88	H ₂ O	173	C ₁₆ H ₂₁ BrN ₄ O ₂
48	CH ₃	CH ₂ CH(CH ₃) ₂	CH ₃	(CH ₂) ₂ Br	A	72	H ₂ O	87	C ₁₆ H ₂₃ BrN ₄ O ₂
49	H	CH ₂ CH ₂ CH ₃	H	(CH ₂) ₂ Br	A	39	H ₂ O	233	C ₁₆ H ₁₉ BrN ₄ O ₂
50	H	CH ₂ CH(CH ₃) ₂	H	(CH ₂) ₂ Br	A	82	H ₂ O	282-284	C ₁₁ H ₁₃ BrN ₄ O ₂
51	CH ₃	CH ₂ CH(CH ₃) ₂	H	CHOH-CH ₂ Br	B	94	CH ₂ Cl ₂	168	C ₁₂ H ₁₇ BrN ₄ O ₃
52	H	CH ₂ CH(CH ₃) ₂	H	CHOH-CH ₂ Br	B	74	CH ₂ Cl ₂ MeOH	250	C ₁₁ H ₁₅ BrN ₄ O ₃

(a) MeOH: methanol; isoPrOH: isopropanol; cyclo H: cyclohexane.

(b) See the corresponding footnotes in Table VII.

(c) See the corresponding footnotes for Table VII. Most of bromalkyl derivatives (Table IV) are unstable and have been very difficult to purify. Some of them (36, 39, 40, 42 and 50) have been used in the raw state.

(d) Prepared starting from No. 28 by reduction with H₂ under 6 atm pressure, in the presence of Raney Ni, in ethanol.

Table V. Effects of 57 on 48/80 induced mast cell degranulation *in vitro*.

Compounds	n	IC ₅₀ ^a (mol/l)
57	9	1.8 × 10 ⁻⁴
Theophylline	5	1.9 × 10 ⁻³
Cromoglycate	4	1 × 10 ⁻³ ^b
Oxatomide	—	0.5–1 × 10 ⁻³ (14)
Mepyramine	1	10 ⁻⁴ = potentiation

^aIncubation for 15 min.^bIncubation for 1 min. No activity after 15 min of incubation.

n = Number of experiments.

Table VI. Protective activity of test compounds against contraction of *taenia coli* induced by CaCl₂.

Drug	n	IC ₅₀	Papaverine ratio
Verapamil	4	0.12 ± 0.03 × 10 ⁻³ M	75
Papaverine	4	9.03 ± 1.79 × 10 ⁻⁴ M	1
Flunarizine	4	6.72 ± 3.63 × 10 ⁻⁴ M	1.35
Compound 57	4	4.73 ± 0.19 × 10 ⁻⁴ M	1.91
Euprotyline	3	1.14 ± 0.06 × 10 ⁻³ M	0.0079
Aminophylline	3	1.43 ± 0.47 × 10 ⁻³ M	0.0062

The IC₅₀ are the means ± SEM of 3–4 experiments for each compound. Asterisks indicate significant differences from papaverine activity (*p* < 0.05) according to the Mann and Whitney test.

potent inhibitors of PDE than AP, and show anti-bronchoconstrictive effects 20- to 80-fold higher than AP. The anti-histaminic drug, mepyramine, is completely devoid of any inhibitory effect on PDE, or PDE + calmodulin enzymatic activity.

Although the inhibitory effects of methylxanthines on phosphodiesterase activity was initially considered as an important mechanism in their anti-asthmatic activities [11], this mechanism remains controversial, since the inhibitory effect requires molecular concentrations higher than the therapeutic concentrations obtained in biological fluids [12]. Nevertheless, it is conceivable that the anti-bronchoconstrictive effect of 57 in guinea pigs depends partly on its inhibitory activity of the PDE involved in reinforced relaxation of the bronchial smooth muscle. Although the IC₅₀ of PDE inhibitory effects of these products are at the same level as the micromolar concentration ranges for which the alkylxanthines have the capability of trigger behavioral effects [13], it is surprising that most of these compounds and mainly 57 are devoid of CNS stimulating effects.

This lack of stimulating actions of 57 can be explained on the one hand, by the poor affinity of this product for the purinergic receptor sites A₁ (IC₅₀ > 10⁻⁵ M) [15] located in the brain cellular membranes, and, on the other hand, by the absence of 57 and its metabolites (J. P. Volland and D. Serkiz, unpublished results) in the cerebral spinal fluid of the dog given progressive doses from 50 to 1000 mg/kg for 6 weeks. This fact was confirmed by autoradiographic analysis of the brain of the rat given [¹⁴C]-labeled 57 at the 8-position (from CEA, Saclay, France). The lack of the

capability of this substance to cross the blood-brain barrier is not yet elucidated and remains under investigation.

On the basis of the results presented here, it can be expected that compound 57 will be a safe and effective drug in the prevention and treatment of asthma in man.

Experimental protocols

Chemistry

Unless otherwise stated, the melting points were determined on a Kofler Block and are uncorrected. IR (Perkin–Elmer model 682) NMR (Bruker model WP 60), UV (Perkin–Elmer model 402) and mass (MAT model 1123) spectra of all compounds were evaluated as being completely consistent with assigned structures. Elemental analyses were performed on Carlo Erba (model 1106) or Perkin–Elmer (Model 240).

All purifications of the compounds listed in Tables I and II were performed by means of FLASH–chromatography glass column with Merck silicagel grade 60 (230–400 mesh) as absorbent, under nitrogen pressure of 0.5–0.8 atm.

Starting materials were prepared following literature methods: 1-isobutyl-3-methyl-6-amino uracil: mp: 90°C [6]; 1-allyl-3-methyl-6-amino uracil: mp: 139°C; 1-phenyl-3-methyl-6-amino uracil: mp: 263–273°C; 1-isobutyl-3-ethyl-6-amino uracil: amorphous; 1-benzyl-6-amino uracil: mp: 260–270°C; 1-isobutyl-6-amino uracil: mp: 267–270°C; 1-isobutyl-3-methyl-5-nitroso-6-amino uracil: mp: 228°C [7]; 1-allyl-3-methyl-5-nitroso-6-amino uracil: mp: 228°C; 1-phenyl-3-methyl-5-nitroso-6-amino uracil: mp: 255°C; 1-isobutyl-3-ethyl-5-nitroso-6-amino uracil: mp: 202°C; 1-benzyl-5-nitroso-6-amino uracil: mp: 243–244°C; 1-isobutyl-5-nitroso-6-amino uracil: mp: 232°C; 1-methyl-3-isobutyl-8-(1-hydroxyethyl)xanthine: mp: 234°C [5]; 1-methyl-3-isobutyl-8-acetylxanthine: mp: 220°C; 1-methyl-3-isobutyl-8-(2-bromooctyl)xanthine: mp: 222°C; 3-methoxypropionic acid: bp: 102–105°C (16 Torr); 4-methoxybutyric acid: bp: 116–120°C (16 Torr) [8].

The *N*-benzylidene *N,N'*-diethylethylenediamine bp: 160–165°C (0.2 Torr) was prepared by heating, under reflux, diphenylchloromethane with an excess of *N,N'*-diethylethylenediamine. The 1-(bis *p*-fluorobenzylidene)piperazine mp: 90–92°C and 1-benzylidene piperazine mp: 91–92°C were prepared starting from respectively, bis (*p*-fluorophenyl) bromomethane and diphenylbromomethane by heating under reflux with an excess of anhydrous piperazine in ethanol.

The 4-(bis *p*-fluorobenzylidene) piperidine mp: 114°C was prepared by reduction under 6 atm H₂ pressure in the presence of 10% Pd on carbon in acetic acid, of 4-(bis *p*-fluorobenzylidene) piperidine, hydrochloride mp: 207–209°C which was prepared through dehydration in conc. HCl, under reflux of bis (*p*-fluorophenyl)(4-piperidyl) methanol hydrochloride mp: 247°C [9].

The 4-benzylideneoxypiperidine bp: 163–170°C (0.3 Torr) was synthesized by hydrolysis with an NaOH–ethanol mixture, of 1-acetyl-4-benzylideneoxypiperidine which was prepared starting from 1-acetyl-4-hydroxypiperidine and diphenylbromomethane, in DMF, in the presence of NaH.

The reported compounds listed in Tables I, II, IV and VII were synthesized by methods identical those described below.

1-Isobutyl-3-methyl-5,6-diamino uracil 2

A solution of 40 g (177 mmol) of 1-isobutyl-3-methyl-5-nitroso-6-amino uracil (mp: 228°C) in 1 l of methanol, was stirred under 7 atm H₂ pressure over 15 g of Raney–NiW-2. After completion (4 h) of hydrogenation, the catalyst was removed by filtration under nitrogen and the solvent evaporated *in vacuo*. The crystals were dried overnight in a vacuum desiccator, at 50°C. Yield: 33.2 g (94%); mp: 174°C.

¹H NMR (DMSO-*d*₆) δ (ppm): 6.1 (broad, 2H, NH₂, exchangeable); 3.7 (d, 2H, N–CH₃); 3.2 (s, 3H, N–CH₃); 2.9 (broad, 2H, NH₂, exchangeable); 2.0 (m, 1H, CH(CH₃)); 0.9 (d, 6H, CH(CH₃)).

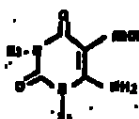
Method A.

1-Isobutyl-3-methyl-5-(3-methoxypropionamide)-6-amino uracil 11

A mixture of 22 g (104 mmol) of 2 and 40 g of 3-methoxypropionic

147
197

Table VII. 1,3-Disubstituted-4,5-diamino-uracils.



N ^o	R ₁	R ₂	R	Yield %	cryst solvent	mp (°C)	FORMULA
1	CH ₃	CH ₃	H	94	MeOH	114-116	C ₁₀ H ₁₀ N ₄ O ₂
2	CH ₃	CH ₂ CH(CH ₃) ₂	H	94	MeOH	174	C ₁₄ H ₁₈ N ₄ O ₂
3	CH ₃	CH ₂ CH = CH ₂	H	96	MeOH	116	C ₁₀ H ₁₀ N ₄ O ₂
4	C ₂ H ₅	CH ₂ CH(CH ₃) ₂	H	99	MeOH	172	C ₁₆ H ₂₂ N ₄ O ₂
5	CH ₃	C ₆ H ₅	H	95	EtOH	194-195	C ₁₅ H ₁₄ N ₄ O ₂
6	H	CH ₂ CH ₂ CH ₃	H	87	acetophen		C ₁₁ H ₁₂ N ₄ O ₂
7	H	CH ₂ CH(CH ₃) ₂	H	88	acetophen		C ₁₂ H ₁₄ N ₄ O ₂
8	CH ₃	CH ₃	CO ₂ (CH ₂) ₂ CO ₂	99	Et ₂ O	211	C ₁₈ H ₁₈ N ₄ O ₆
9	CH ₃	CH ₃	CO ₂ (CH ₂) ₃ CO ₂	91	Et ₂ O	204	C ₁₉ H ₁₈ N ₄ O ₆
10	CH ₃	CH ₂ CH(CH ₃) ₂	CO ₂ (CH ₂) ₂ CO ₂	91	acetophen		C ₂₂ H ₂₄ N ₄ O ₆
11	CH ₃	CH ₂ CH(CH ₃) ₂	CO ₂ (CH ₂) ₃ CO ₂	87	Et ₂ O	208	C ₂₃ H ₂₄ N ₄ O ₆
12	CH ₃	CH ₂ CH(CH ₃) ₂	CO ₂ (CH ₂) ₄ CO ₂	85	Et ₂ O	198	C ₂₄ H ₂₄ N ₄ O ₆
13	CH ₃	CH ₂ CH = CH ₂	CO ₂ (CH ₂) ₂ CO ₂	24	acetophen		C ₁₈ H ₁₈ N ₄ O ₆
14	C ₆ H ₅	CH ₂ CH(CH ₃) ₂	CO ₂ (CH ₂) ₂ CO ₂	95	acetophen		C ₂₂ H ₂₂ N ₄ O ₆
15	CH ₃	C ₆ H ₅	CO ₂ (CH ₂) ₂ CO ₂	84	acetophen		C ₁₉ H ₁₈ N ₄ O ₆
16	H	CH ₂ CH ₂ CH ₃	CO ₂ (CH ₂) ₂ CO ₂	99	Et ₂ O	190	C ₁₅ H ₁₆ N ₄ O ₆
17	H	CH ₂ CH(CH ₃) ₂	CO ₂ (CH ₂) ₂ CO ₂	98	Et ₂ O	188	C ₁₆ H ₁₈ N ₄ O ₆

(x) Commercial product purchased from Aldrich Co.
 (a) MeOH: methanol; Et₂O: ethyl ether.
 (b) K: Kofler block; esp: Mel-Temp capillary apparatus.
 (c) Most of compounds have been purified by column chromatography and most of them, analyzed for C, H, N with results (± 0.4% limit) by means of Perkin-Elmer autoanalyzer 240. However, some diamino derivatives (Table VII) have not been very purified and analyzed on account of their instability (No. 6, 7, 10, 16, 17). The others have been analyzed with results for the N value out of the 0.4% limit.

acid was stirred and heated at 100°C for 4 h. After cooling at 20°C, the solution was stirred for 20 h and treated with H₂O (300 ml). The resulting crystallized product was filtered off, washed with H₂O and dried in a vacuum desiccator at 50°C. Yield: 26.9 g (87%); mp: 200°C.

1-Methyl-3-isobutyl-8-(2-methoxyethyl)xanthine 23
 A solution of 2.9 g (10 mmol) of 11 in 12 ml of H₂O and 1 ml of 12 N NaOH, was heated at 100°C for 2 h. After cooling at 5°C, the solution was made acidic (pH 6) by the addition of 4 N H₂SO₄. The precipitate was filtered off, washed with H₂O and dried in a vacuum desiccator at 50°C. Yield: 2.3 g (89%); mp: 165°C.
¹H NMR (CDCl₃) δ (ppm): 4.0 (d, 2H, N—CH₃); 3.9 (t, 2H, CH₂O); 3.5 (s, 3H, OCH₃); 3.4 (s, 3H, N—CH₃); 3.2 (t, 2H, CH₂CH₂O); 2.4 (m, 1H, CH(CH₃)₂); 1.0 (d, 6H, CH(CH₃)₂).

1,7-Dimethyl-3-isobutyl-8-(2-methoxyethyl)xanthine 25
 Methyl iodide (3.1 ml) was gradually added at 20°C to a solution of 26.6 g (95 mmol) of 23 in 190 ml of DMF, in the presence of K₂CO₃ (13 g). Then, the mixture was progressively heated at 50°C. After 1 h, methyl iodide (3.1 ml) was added again. After heating for another 2 h, the potassium salt was filtered off and the solution was evaporated *in vacuo*. The crude product was recrystallized in cyclohexane. Yield: 24.9 g (89%); mp: 82°C.
¹H NMR (CDCl₃) δ (ppm): 3.95 (s, 3H, N—CH₃); 3.9 (d, 2H, N—CH₂CH); 3.8 (t, 2H, CH₂O); 3.4—3.35 (s, s, 6H, N—CH₃, OCH₃); 3.0 (t, 2H, CH₂CH₂O); 2.3 (m, 1H, CH(CH₃)₂); 0.9 (d, 6H, CH(CH₃)₂).

1,7-Dimethyl-3-propyl-8-(3-methoxypropyl)xanthine 29
 A solution of 4 g (13.7 mmol) of 28 in 100 ml of ethanol was stirred for 3 h at 20°C under 8 atm H₂ pressure in the presence of 0.4 g of 5% Pd on a carbon catalyst. After removing the catalyst by filtration, the

solution was evaporated *in vacuo*. 3.95 g (94%) of pure crystallized product was obtained; mp: 67°C.

¹H NMR (CDCl₃) δ (ppm): 4.7 (t, 2H, N—CH₂); 4.0 (s, 3H, N—CH₃); 3.4 (t, 2H, CH₂O); 3.3—3.35 (s, s, 6H, (O—C)₂N—CH₃, OCH₃); 2.9 (t, 2H, CH₂CH₂CH₂O); 2.4—1.3 (m, 4H, CH₂); 1.0 (t, 3H, CH₃—CH₂).

1-Methyl-3-isobutyl-8-(2-bromoethyl)xanthine 40

A solution of 17.6 g (63 mmol) of 23 in 300 ml of 48% HBr was stirred and heated under reflux for 20 h with slow bubbling of dry HBr into the solution. After completion of the reaction, the solution was evaporated *in vacuo* and the crystallized residue was washed several times with ice water, filtered off and dried in a vacuum desiccator at 60°C. Yield: 17.2 g (83%); mp: 210°C.

Method B

1-Methyl-3-isobutyl-8-(2-bromo-1-hydroxyethyl)xanthine 51

To a stirred solution of 3.43 g (10 mmol) of 1-methyl-3-isobutyl-8-(2-bromoacetyl)xanthine (mp: 220°C) in 240 ml of methanol cooled at 10°C, was added quickly 0.34 g of NaBH₄. After stirring at 5°C for 1 h, 10 ml of N HCl were added and the resulting acidic solution was evaporated *in vacuo*. The crude residue was dissolved in CH₂Cl₂ and washed with H₂O. The solvent was evaporated *in vacuo* and the crystallized product was dried in a vacuum desiccator at 70°C. Yield: 3.25 g (94%); mp: 170°C.

¹H NMR (CDCl₃+DMSO-d₆) δ (ppm): 14 (broad, 1H, NH exchangeable); 5.0 (t, 1H, CHOH); 4.1—3.6 (m, 4H, N—CH₂, CH₂Br); 3.3 (s, 3H, N—CH₃); 2.2 (m, 1H, CH(CH₃)₂); 0.9 (d, 6H, CH(CH₃)₂).

The starting material 1-methyl-3-isobutyl-8-(2-bromoacetyl)xanthine was prepared according to [5] in the following manner: to a stirred solution of 18.15 g (68.7 mmol) of 1-methyl-3-isobutyl-8-acetylxanthine (mp: 222°C) in 170 ml of acetic acid heated under reflux, was gradually added a solution of 34.1 g of pyridosone hydrotribromide in 140 ml of acetic acid. The resulting solution was heated for 1.5 h under reflux and after cooling, was poured into 2 l of water. The crystals were filtered off, washed with H₂O and dried in a vacuum desiccator at 20°C. Yield: 19.3 g (82%); mp: 220°C.

1-Methyl-3-isobutyl-8-[2-(4-benzhydryl piperazine)ethyl]xanthine 57

A solution of 13.2 g (40 mmol) of 40 and 25.2 g (100 mmol) of 4-benzhydrylpiperazine in 200 ml of anhydrous methanol was stirred and heated under reflux for 20 h. After removing the solvent *in vacuo*, the crude residue was treated with CH₂Cl₂ (300 ml) and 10% NaHCO₃ solution (300 ml) and the mixture was stirred for 0.5 h. After drying (Na₂SO₄) the organic extract and removing the solvent *in vacuo*, the crude crystallized product (22 g) was purified by chromatography (1.2 kg of silica gel; ethyl acetate). Yield: 14.4 g (72%); mp: 196—197°C.

¹H NMR (CDCl₃) δ (ppm): 12.5—9.5 (broad, 1H, NH exchangeable); 7.5—7.0 (m, 10H, 2C₆H₅); 4.21 (s, 1H, N—CH₂); 3.9 (d, 2H, N—CH₂—CH); 3.4 (s, 3H, N—CH₃); 3.0—2.9 (m, 4H, CH₂—CH₂—N); 2.7—2.4 (s, 4H, signals, 8H, piperazine); 2.3 (m, 1H, CH(CH₃)₂); 0.9 (d, 6H, CH(CH₃)₂).

The first fractions of the chromatography contained the crystallized product 57b (1.5 g), mp: 223°C, which was isolated after removing the solvent *in vacuo*.

1-Methyl-3-isobutyl-7-hydroxyethyl-8-[2-(4-benzhydryl piperazine)ethyl]xanthine 72

To a stirred solution of 10 g (20 mmol) of 57 in 200 ml of anhydrous DMF, heated at 110°C, and containing 37.6 g of K₂CO₃, 30 ml of 2-chloroethanol were added quickly.

After heating at 110°C for 1 h, another 30 ml of 2-chloroethanol and 37.6 g of K₂CO₃ were added to the mixture and heating at 110°C was continued for 1.5 h. After the completion of the reaction, the salt was filtered off and the solution was evaporated *in vacuo*. The crude residue was treated with CH₂Cl₂ (200 ml) and water and the organic solutions were dried on MgSO₄. After removing the solvent *in vacuo*, the raw compound (12 g) was purified by chromatography (750 g of silica gel; ethyl acetate—methanol (95—5)). The crystals obtained after removing the solvent, were dissolved in 50 ml of isopropanol. After the addition of an HCl—ether solution and crystallization, 8.75 g (75%) of crystals were isolated mp (cap): 190—200°C.

¹H NMR (DMSO-d₆) δ (ppm): 8.0–6.0 (broad, 2H, ²NH, exchangeable); 8.0 (m, 4H, *ortho* H); 7.5 (m, 6H, *meta* and *para* H); 5.9 (broad, 1H, N—CH); 4.4 (m, 2H, CH₂OH); 4.2–3.0 (set of signals, 16H, CH₂); 3.3 (s, 3H, N—CH₃); 2.3 (m, 1H, CH(CH₃)); 0.9 (d, 6H, CH(CH₃)₂).

Pharmacological methods

Protection against histamine-induced bronchoconstriction in the guinea pig
Male Hartley guinea pigs weighing 375–400 g were placed in a plastic chamber and exposed to a histamine aerosol (0.4% w/v) produced by a Halpern column and a compressed air supply. The preconvulsion times two days before the administration of the test compound were determined. The protective effect of the drug was evaluated in guinea pigs, by exposing them to the histamine aerosol 45 min (sometimes 24 or 48 h) after oral administration. Recovery of the preconvulsion time to the control value was verified 48 and 96 h after treatment. The percentage of protection was assessed according to Armitage *et al.* [10].

Phosphodiesterase inhibition

Enzyme was prepared from whole brains of adult Sprague–Dawley rats homogenized under cold conditions. After centrifugation, phosphodiesterase activity in the supernatant was determined according to the spectrophotometric method [11].

Rat mast cell test

Male Sprague–Dawley rats (weighing 300–350 g) were anesthetized with Nembutal®. 10 ml of Tyrode medium were injected into the abdominal cavity. After 2 min of gentle massage, the abdominal fluid was collected and centrifuged at 300 × g for 5 min at room temperature. After washing, the cell pellet was suspended in Tyrode solution. The cell suspension was then incubated for 10 min at 37°C with compound 48/80 and test compounds. Control experiments were performed without compound 48/80 or test compounds. Degranulation of mast cells was observed microscopically and the activity of the test compounds was expressed as the IC₅₀ value (concentration of the drug required to inhibit the 48/80 induced degranulation by 50%).

Spasmolytic activity

Male Hartley guinea pigs weighing 375–400 g were killed by a blow to the head, then strips of *taenia coli* were rapidly dissected. The preparations were immediately suspended in an organ bath containing oxygenated Tyrode solution. After an equilibration period, membrane depolarization was provoked by replacing the tyrode solution with

Ca free K₂SO₄ Ringer's solution. The inhibiting effect of the test drug on the Ca-induced contraction of *taenia coli* was studied by adding CaCl₂–KNO₃ Ringer's solution to the organ bath. The contractile force was obtained from the amplitude of the *taenia coli* contractions. The IC₅₀ was defined as the concentration of test compound required to reduce by 50% the maximal contraction obtained with Ca. IC₅₀ values were obtained from the individual dose–response curves. Calculation of means ± SEM and statistical comparison of IC₅₀ values were carried out using the Mann and Whitney test.

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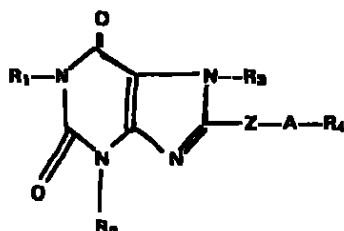
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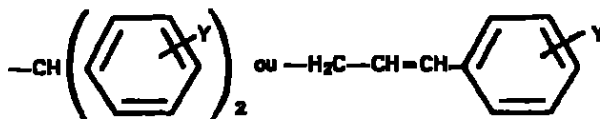
82 Nouveaux dérivés de la xanthine, leur procédé de préparation et les compositions pharmaceutiques les contenant.

Nouveaux dérivés de la xanthine, utilisables comme médi-
caments, et répondant à la formule:



dans laquelle:

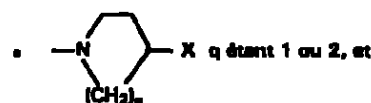
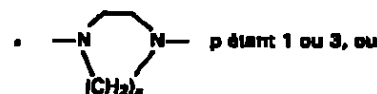
R₁ est hydrogène ou un alkyle de C₁ à C₆;
R₂ est un radical hydrocarboné jusqu'en C₆ ayant éventuelle-
ment une double liaison, un radical phényle, ou un radical
benzyle.
R₃ est hydrogène, alkyle, hydroxyalkyle ou dihydroxyalkyle
chacun de C₁ à C₆;
R₄ représente



Y étant hydrogène, halogène, alkyle ou alkoxy de C₁ à C₆, ou
hydroxyle;

Z est méthylène ou un radical hydrocarboné en chaîne droite ou
ramifiée de C₂ à C₆, éventuellement substitué par un hydro-
xyle; et

A est un reste aminé de formule:

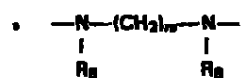


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X étant une liaison simple, oxygène ou —N— , R_5 étant hydrogène, alkyle ou alkylène jusqu'en C_6 , ou



dans lequel:

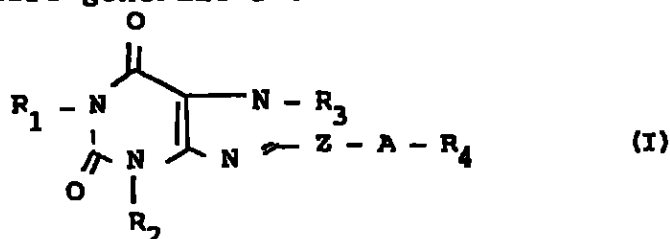
m est un nombre entier de 2 à 6 et R_5 est alkyle de C_1 à C_6 .

Ces nouveaux composés et leurs sels physiologiquement tolérables peuvent être utilisés en thérapeutique notamment dans le traitement de toutes les maladies où il est nécessaire d'inhiber les réactions antigène anticorps.

NOUVEAUX DERIVES DE LA XANTHINE, LEUR PROCEDE DE
PREPARATION ET LES COMPOSITIONS PHARMACEUTIQUES
LES RENFERMANT

La présente invention a pour objet de nouveaux
dérivés de la xanthine, leur procédé de préparation et
les compositions pharmaceutiques les renfermant.

Elle concerne particulièrement les dérivés de
5 la xanthine de formule générale I :



dans laquelle :

- R₁ représente un atome d'hydrogène ou un radical alkyle en chaîne droite ou ramifiée, renfermant 10 de 1 à 5 atomes de carbone ;
- R₂ représente un radical hydrocarboné en chaîne droite ou ramifiée renfermant jusqu'à 6 atomes de carbone, et comportant éventuellement une double liaison, un radical phényle, ou un radical benzyle ;
- 15 - R₃ représente un atome d'hydrogène ou un radical alkyle, hydroxyalkyle ou dihydroxyalkyle

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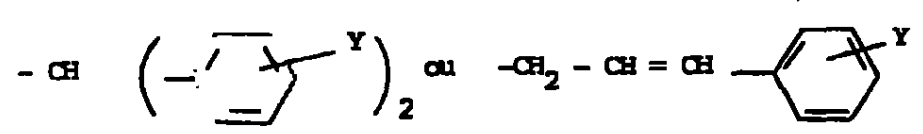
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renfermant chacun de 1 à 5 atomes de carbone ;

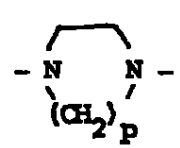
- R_4 représente un groupe de formule :



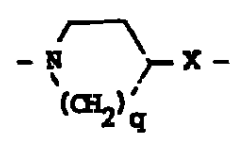
dans lesquelles Y représente un atome d'hydrogène ou d'halogène ou un radical alkyle ou alkoxy renfermant chacun de 1 à 5 atomes de carbone, ou un radical hydroxyle ;

- Z représente un radical méthylène ou un radical hydroxycarboné en chaîne droite ayant de 2 à 5 10 atomes de carbone éventuellement substitué par un radical hydroxyle ; et

- A représente un reste aminé de type :



dans lequel p prend les valeurs 2 ou 3, ou



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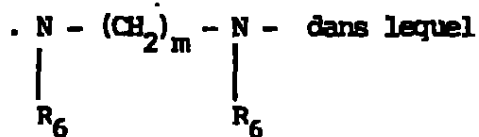
dans lequel :

q prend les valeurs 1, ou 2, et

X représente une liaison simple, un atome d'oxygène ou un groupe - N - dans lequel



R_5 représente un atome d'hydrogène ou un radical alkyle ou alkylène ayant chacun de 1 à 5 atomes de carbone ; ou

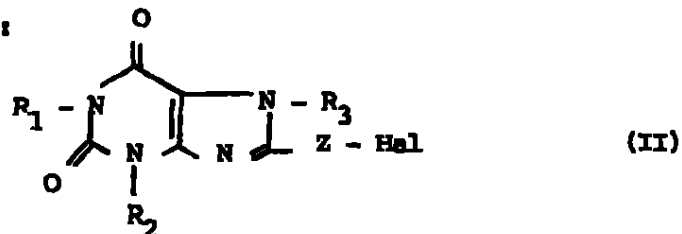


5 m représente un nombre entier de 2 à 6 et,

R_6 représente un radical alkyle renfermant de 1 à 5 atomes de carbone.

La présente invention a également pour objet le procédé de préparation des dérivés de formule générale I, 10 caractérisé en ce que :

- l'on condense un dérivé halogéné de formule générale II :



dans laquelle R_1 , R_2 , R_3 et Z ont les significations 15 précédemment définies et Hal représente un atome de chlore ou de brome,

- avec un dérivé aminé de formule générale III

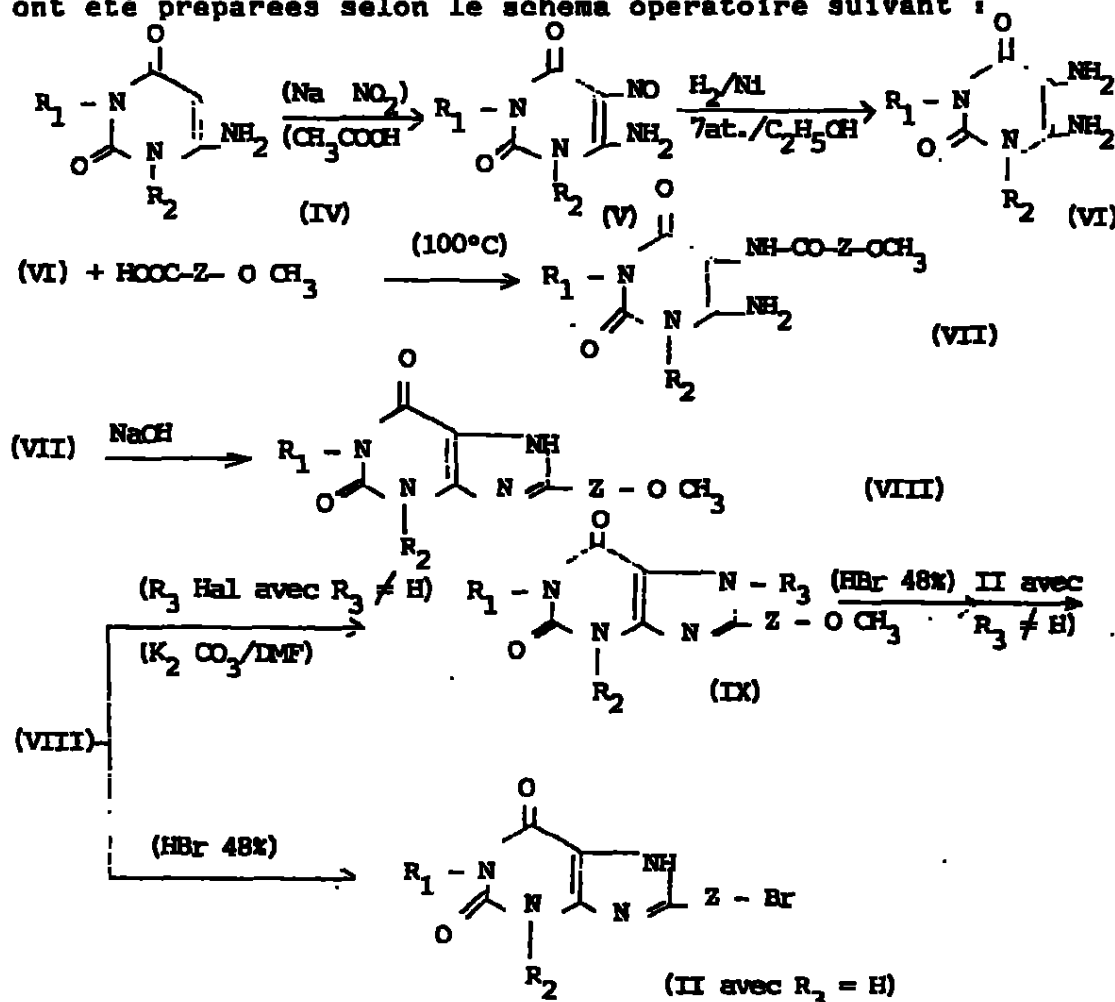


dans laquelle A et R_4 sont tels que précédemment 20 définis.

La condensation s'effectue de préférence dans un solvant choisi parmi les alcools renfermant jusqu'à 5

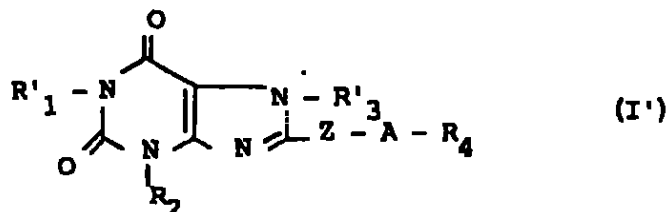
atomes de carbone, tel que par exemple le méthanol, l'éthanol, le propanol ou le butanol. Il est avantageux d'opérer à une température comprise entre 64 et 130°C, en présence d'un accepteur de l'hydraide formé au cours de la réaction. Cet accepteur peut-être choisi parmi les carbonates alcalins tels que les carbonates de sodium et de potassium, les amines tertiaires telles que la triéthylamine ou un excès du dérivé aminé de formule III utilisé pour la réaction.

10 Les matières premières de formule générale II ont été préparées selon le schéma opératoire suivant :



Les matières premières de formule générale III sont des produits connus.

La présente invention a également pour objet le procédé de préparation des dérivés (I) répondant à la formule générale I' :



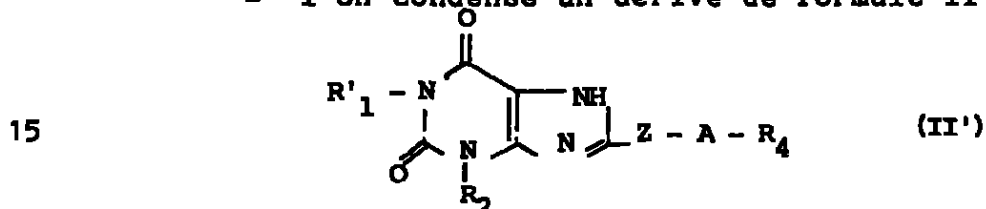
5 dans laquelle :

- R_2 , R_4 , Z et A ont les significations précédemment définies,

- R'_1 représente un radical alkyle en chaîne droite ou ramifiée, renfermant de 1 à 5 atomes de carbone
10 et

- R'_3 représente un radical alkyle, hydroxy-alkyle ou dihydroxyalkyle contenant chacun de 1 à 5 atomes de carbone ; caractérisé en ce que :

- l'on condense un dérivé de formule II' :



dans laquelle R'_1 , R_2 , R_4 , Z et A ont les significations précédemment définies,

avec un dérivé halogéné de formule III' :



20 dans laquelle R'_3 a la signification énoncée précédemment et X représente un atome de chlore ou de brome.

La condensation s'effectue de préférence dans un solvant adéquat tel que par exemple, le diméthylformamide à une température comprise entre 80 et 120°C, en présence d'un accepteur de l'hydracide formé au cours de la réaction. Cet accepteur peut-être, entre autre, un carbonate alcalin comme par exemple le carbonate de sodium ou de potassium.

Les nouveaux dérivés de formule générale I peuvent être transformés en sels d'addition avec les acides, sels qui font à ce titre, partie de l'invention. Comme acides utilisables pour la formation de ces sels, on peut citer par exemple dans la série minérale : les acides chlorhydrique, bromhydrique, sulfurique, phosphorique, et dans la série organique, les acides acétique, propionique, maléique, fumarique, tartrique, citrique, oxalique, benzoïque, méthane sulfonique et iséthionique.

Ces nouveaux dérivés peuvent être purifiés par des méthodes physiques telles que cristallisation, chromatographie ou chimiques telles que formation de sels d'addition avec des acides et décomposition de ces sels par les agents alcalins.

Les dérivés de formule générale I et leurs sels physiologiquement tolérables possèdent des propriétés pharmacologiques et thérapeutiques intéressantes, notamment des propriétés bronchodilatatrice, anti-allergique et inhibitrice de la phosphodiesterase.

Leur toxicité est faible et leur D L 50 déterminée chez la souris est supérieure à 100 mg/kg par voie intrapéritonéale, et supérieure à 800 mg/kg par voie orale.

L'activité bronchodilatatrice a été étudiée chez le cobaye par la méthode de H. KONZETT et R. ROSSLER

Arch. Exp. U. Pharm. 195, 71 (1940). On a ainsi observé que les dérivés de la présente invention injectés par voie intraveineuse à des doses variant, selon les dérivés de 1 à 5 mg/kg inhibent totalement le bronchospasme provoqué par l'administration intraveineuse soit d'histamine, soit de sérotonine, et partiellement l'effet de l'acétylcholine et de la Slow Reacting Substance.

Soumis au test de A. K. Armitage, Brit. J. Pharmacol. 17, 196, (1961), les dérivés de l'invention administrés par voie orale à des doses variant de 0,5 à 10 mg/kg selon les composés, inhibent de 50 % l'effet produit chez le cobaye par un aérosol d'histamine à 4 %. Pour certains de ces composés, l'effet est encore très important 48 heures après l'administration orale.

15 En outre, certains composés de l'invention possèdent un effet agoniste spécifique vis à vis des récepteurs purinergiques centraux et périphériques de type A 1 et/ou A 2 qui peut conduire à une spécificité d'effet thérapeutique.

20 A titre d'exemple non-limitatif, l'administration orale de 5 mg/kg du composé de l'exemple 1 inhibe, pendant plus de 48 heures, le bronchospasme provoqué par un aérosol d'histamine à 4 %. Par ailleurs une diminution de la réaction cutanée anaphylactique est
25 observée chez le rat après l'administration unique de 20 mg/kg per os de ce composé.

Ce composé de l'exemple 1 est plus fortement agoniste des récepteurs A 2 ($IC_{50} = 15 \mu M$) que des récepteurs A 1 ($IC_{50} > 100 \mu M$).

30 Les propriétés pharmacologiques ci-dessus décrites, ainsi que la faible toxicité des dérivés de formule générale I et de leurs sels physiologiquement

tolérables permettent leur utilisation en thérapeutique notamment dans le traitement de toutes les maladies où il néoessaire d'inhiber les réactions antigène - anticorps commes les maladies autoimmunes, allergiques et inflam-
5 matoires et en particulier celles dans lesquelles un effet bronchodilatateur est bienvenu telles que la dyspnée asthmatique et les bronchopneumopathies chro-
niques obstructives, en particulier à forme spastique. La longue durée d'action permet un traitement de crise aussi
10 bien qu'un traitement de fond de la maladie asthmatique simple ou intriguée.

Par ailleurs, les propriétés spasmolytiques sont une indication dans le traitement des coliques néphrétiques ou hépatiques.

15 La présente invention a également pour objet les compositions pharmaceutiques contenant comme principe actif un dérivé de formule générale I, ou un de ses sels physiologiquement tolérables, mélangé ou associé à un excipient pharmaceutique approprié. Elle concerne notam-
20 ment les formes dosées renfermant de 25 à 250 mg de principe actif.

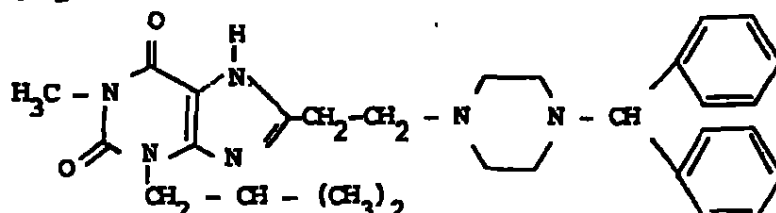
Les compositions pharmaceutiques ainsi obtenues, sont présentées avantageusement sous des formes dosées diverses telles que par exemple comprimés,
25 dragées, gélules, glosesettes ou préparations galéniques appropriées pour une administration sublinguale, suppositoires, solutions injectables ou buvables, ainsi que sous des formes adaptées à l'administration par aérosol. Elles peuvent être administrées par la voie orale, rec-
30 tale, ou parentérale aux doses de 25 à 250 mg de principe actif, 1 à 2 fois par jour.

Les exemples suivants, donnés à titre non-limitatif, illustrent l'invention. Les points de fusion

sauf mention contraire, ont été déterminés à la platine chauffante de KOFLER.

EXEMPLE 1 :

méthyl-1 isobutyl-3 [(diphénylméthyl-4 pipérazine-5 zinyl)-2 éthyl]-8 xanthine.



Une suspension de 12,2 g de méthyl-1 isobutyl-3 bromoéthyl-8 xanthine et de 23 g de benzhydryl-1 pipérazine dans 200 ml d'éthanol est chauffée à reflux. On observe une dissolution progressive et on chauffe ainsi pendant 20 à 24 heures. On évapore ensuite à sec et reprend le résidu par une solution à 10 % de bicarbonate de sodium. On extrait plusieurs fois avec CH_2Cl_2 , sèche sur Na_2SO_4 et concentre à sec. Le résidu huileux est purifié par chromatographie flash sur 1 kg de silice 0,04-0,063 mm, en éluant avec de l'acétate d'éthyle puis avec un mélange d'acétate d'éthyle méthanol (95/5). Après fractionnement et évaporation des éluats, on recueille 14 g de produit pur fondant à 200-202° C.

La méthyl-1 isobutyl-3 bromoéthyl-8 xanthine de départ, fondant à 210° C, a été préparée par bromuration avec H Br à 48 % du dérivé méthoxy éthyl-8 correspondant, fondant à 163° C, lui-même préparé par cyclisation avec Na OH de 1'isobutyl-1 méthyl-3 dioxo-2,4 tétrahydro-1, 2, 3, 4 (méthoxy-3 propionamido) -5 amino-6 pyrimidine fondant à 200° C, elle-même préparée par condensation de l'acide méthoxy-3 propionique avec la diamino-5,6 isobutyl-1 méthyl-3 dioxo-2,4 tétrahydro-1, 2, 3, 4, pyrimi-

dine, elle-même préparée par réduction, en présence de nickel de Raney comme catalyseur, sous une pression d'hydrogène de six atmosphères, du dérivé nitroso-5 correspondant fondant à 228° C, lui-même préparé par nitrosation avec $\text{Na NO}_2/\text{CH}_3\text{CO OH}$ de l'isobutyl-1 méthyl-3 dioxo-2,4 amino-6 tétrahydro-1, 2, 3, 4, pyrimidine.

EXEMPLE 2 à 30 :

Les dérivés suivants ont été préparés selon le procédé décrit dans l'exemple 1 :

- 10 2) La diméthyl-1,3 (diphénylméthyl-4 pipérazinyl méthyl)-8 xanthine, P. F. : 241° C (méthanol)
- 3) La diméthyl-1,3 [(diphénylméthyl-4 pipérazinyl) -2 éthyl] -8 xanthine, P. F. (capillaire) : 103-106° C (chlorure de méthylène).
- 15 4) La diméthyl-1,3 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine, P. F. (capillaire) du dichlorhydrate hémihydrate correspondant : 249-250° C (méthanol)
- 5) La méthyl-1 isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine, P. F. (capillaire) du dichlorhydrate monohydrate correspondant : 217-220° C (éthanol).
- 20 6) La triméthyl-1, 3, 7 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine, P. F. du fumarate 25 correspondant : 198° C (éthanol)
- 7) La diméthyl-1,7 isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine, P. F. du fumarate correspondant : 182° C (éthanol/éther)

- 8) La diméthyl-1,7 isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, P. F. (capillaire) du dichlorhydrate correspondant : 214-218° C (n. propanol-éther)
- 5 9) La diméthyl-1,7 phényl-3 [(diphénylméthyl-4 pipérazinyl)-3 propyl]-8 xanthine, P. F. : 150° C (isopropanol)
- 10 10) La méthyl-1 isobutyl-3 [(diparafluorophénylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, P. F. : 184° C (acétate d'éthyle)
- 11) La diméthyl-1,7 isobutyl-3 [(diparafluorophénylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, P. F. du dimaléate correspondant : 174° C (n. propanol)
- 15 12) La diméthyl-1,7 isobutyl-3 {[(diparafluorophénylméthyl)-4 pipérazinyl]-3 propyl}-8 xanthine, P. F. du fumarate correspondant = 180° C (éthanol)
- 20 13) La méthyl-1 isobutyl-3 { [N-(N'-diphénylméthyl N'-éthylamino)-2 éthyl] N-éthylamino-2 éthyl } -8 xanthine, P. F. (capillaire) du dichlorhydrate correspondant : 135-140° C (isopropanol/éther)
- 14) La diméthyl-1,7 isobutyl-3 { [N-(N'-diphénylméthyl N'-éthylamino)-2 éthyl] N-éthylamino-2 éthyl } -8 xanthine, P. F. (capillaire) du dichlorhydrate correspondant : 130-140° C (isopropanol/éther)
- 25 15) La diméthyl-1,7 isobutyl-3 { [N-(N'-diphénylméthyl N'-éthylamino)-2 éthyl] N-éthylamino-3 propyl } -8 xanthine, P. F. (capillaire) du dichlorhydrate correspondant : 125-135° C.

- 16) La diméthyl-1,7 n. propyl-3 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine, P. F. du fumarate correspondant : 200° C (éthanol).
- 17) La méthyl-1 isobutyl-3 [(diphénylméthoxy-4 pipéridino)-2 éthyl] -8 xanthine, P. F. (capillaire) du dichlorhydrate correspondant : 163-167° C (isopropanol /éther)
- 18) La diméthyl-1,7 isobutyl-3 [(diphénylméthoxy-4 pipéridino)-2 éthyl] -8 xanthine, P. F. (capillaire) du fumarate correspondant : 173-177° C (n. propanol)
- 19) La diméthyl-1,7 isobutyl-3 [(diphénylméthoxy-4 pipéridino)-3 propyl] -8 xanthine, P. F. du fumarate correspondant : 194° C (éthanol)
- 15 20) La méthyl-1 isobutyl-3 (diphénylméthyl-4 pipérazinyl méthyl)-8 xanthine, P. F. 182° C
- 21) La méthyl-1 isobutyl-3 [(diparafluorophénylméthyl-4 pipérazinyl)-2 éthyl] -8 xanthine, P. F. : 200°C.
- 22) La méthyl-1 isobutyl-3 [(cinnamyl-4 pipérazinyl)-2 éthyl] -8 xanthine, P. F. : 140° C (chlorure de méthylène).
- 23) L'éthyl-1 isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine, P. F. (capillaire) du fumarate correspondant : 201-205° C (n. propanol)
- 25 24) L'éthyl-1 isobutyl-3 méthyl-7 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine, P. F. du maléate correspondant : 193° C (n. propanol)

25) L'isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, P.F. : 240°C (acétate d'éthyle).

26) La benzyl-3 [(diphénylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, P.F. : (capillaire) : 232-235°C
5 (acétate d'éthyle).

27) La méthyl-1 isobutyl-3 (dihydroxy-2,3 propyl)-7 [(diphénylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, P.F. (capillaire) du dichlorhydrate correspondant : 145-155°C (isopropanol/éther).

10 28) La méthyl-1 isobutyl-3 (hydroxy-2 éthyl)-7 [(diphénylméthyl-4 pipérazine)-2 éthyl]-8 xanthine, P.F. (capillaire) du dichlorhydrate correspondant : 190-200°C (isopropanol/éther).

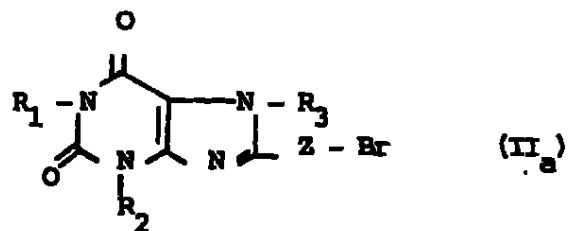
29) La R,S méthyl-1 isobutyl-3 hydroxy-1
15 [(diphénylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, P.F. : 212°C (éther éthylique).

30) L'isobutyl-3 [hydroxy-1 (diphénylméthyl-4 pipérazinyl)-2 éthyl] xanthine, P.F. : 234°C avec décomposition (acétate d'éthyle).

20 Les produits objets des exemples 2 à 30 ont été préparés à partir des composés de formule II_a dont les caractéristiques sont regroupées ci-après dans le tableau (A). Ces produits de formule II_a ont eux-mêmes été préparés selon le procédé objet du schéma opératoire donné
25 précédemment, à partir des composés dont les caractéristiques sont regroupées ci-après dans les tableaux (B), (C) et (D).

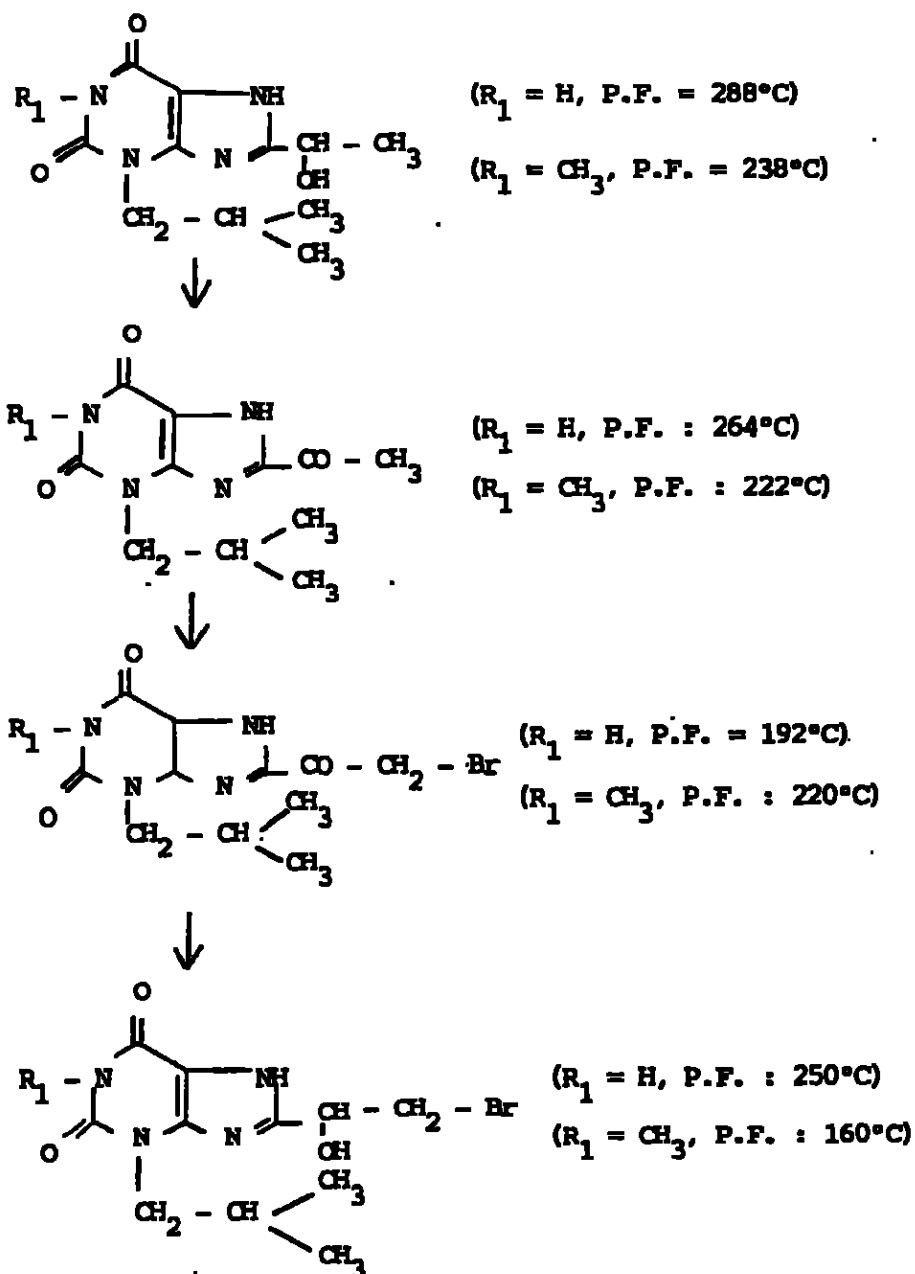
TABLEAU (A)

Dérivés de formule :



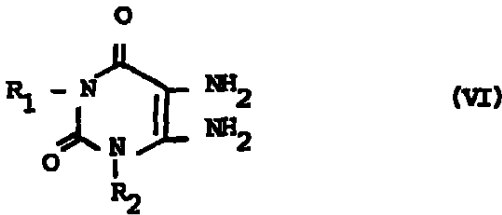
R ₁	R ₂	R ₃	Z	P.F. (Kofler) en °C
CH ₃	CH ₃	H	(CH ₂) ₂	227
CH ₃	CH ₃	H	(CH ₂) ₃	220
CH ₃	CH ₃	CH ₃	(CH ₂) ₃	130
CH ₃	-CH ₂ -CH-(CH ₃) ₂	H	-CH ₂	224
CH ₃	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₂	210
CH ₃	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₃	182
CH ₃	-CH ₂ -CH-(CH ₃) ₂	CH ₃	(CH ₂) ₂	114
CH ₃	-CH ₂ -CH-(CH ₃) ₂	CH ₃	(CH ₂) ₃	100
CH ₃	-CH ₂ -CH ₂ -CH ₃	CH ₃	(CH ₂) ₃	92
CH ₃	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₃	166
CH ₃		CH ₃	(CH ₂) ₃	156
C ₂ H ₅	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₃	172
H	-CH ₂	H	(CH ₂) ₂	252
H	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₂	292-294
*CH ₃	-CH ₂ -CH-(CH ₃) ₂	H	-CH-CH ₂ OH	160
*H	-CH ₂ -CH-(CH ₃) ₂	H	-CH-CH ₂ OH	250

NB : Les dérivés * ont été préparés par analogie avec
G. EHRHAR et coll., Arch. der Pharm. 289, 453-459, (1956),
selon le schéma suivant :



T A B L E A U (B)

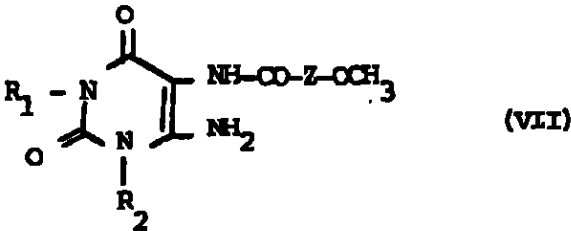
Dérivé de formule :



R ₁	R ₂	P.F. (Kofler) en °C
CH ₃	CH ₃	210-214
CH ₃	-CH ₂ -CH-(CH ₃) ₂	174
CH ₃	-CH ₂ -CH=CH ₂	116
CH ₃	-CH ₂ -CH-(CH ₃) ₂	172
CH ₃		250-252
C ₂ H ₅	-CH ₂ -CH-(CH ₃) ₂	amorphe
H	-CH ₂ - 	243-244
H	-CH ₂ -CH-(CH ₃) ₂	non isolé

T A B L E A U (C)

Dérivé de formule :

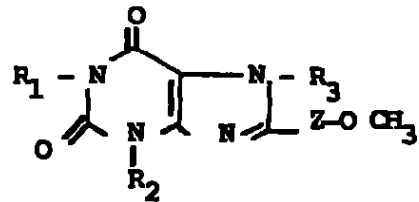


R ₁	R ₂	Z	P.F. (Kofler) en °C
CH ₃	CH ₃	(CH ₂) ₂	208
CH ₃	CH ₃	(CH ₂) ₃	221
CH ₃	-CH ₂ -CH-(CH ₃) ₂	CH ₂	amorphe
CH ₃	-CH ₂ -CH-(CH ₃) ₂	(CH ₂) ₂	200
CH ₃	-CH ₂ -CH-(CH ₃) ₂	(CH ₂) ₃	150
CH ₃	-CH ₂ -CH=CH ₂	(CH ₂) ₃	amorphe
C ₂ H ₅	CH ₂ -CH-(CH ₃) ₂	(CH ₂) ₃	amorphe
CH ₃		(CH ₂) ₃	amorphe
H	-CH ₂ - 	(CH ₂) ₂	190
H	-CH ₂ -CH-(CH ₃) ₂	(CH ₂) ₂	158

TABLEAU (D)

Dérivé de formule :

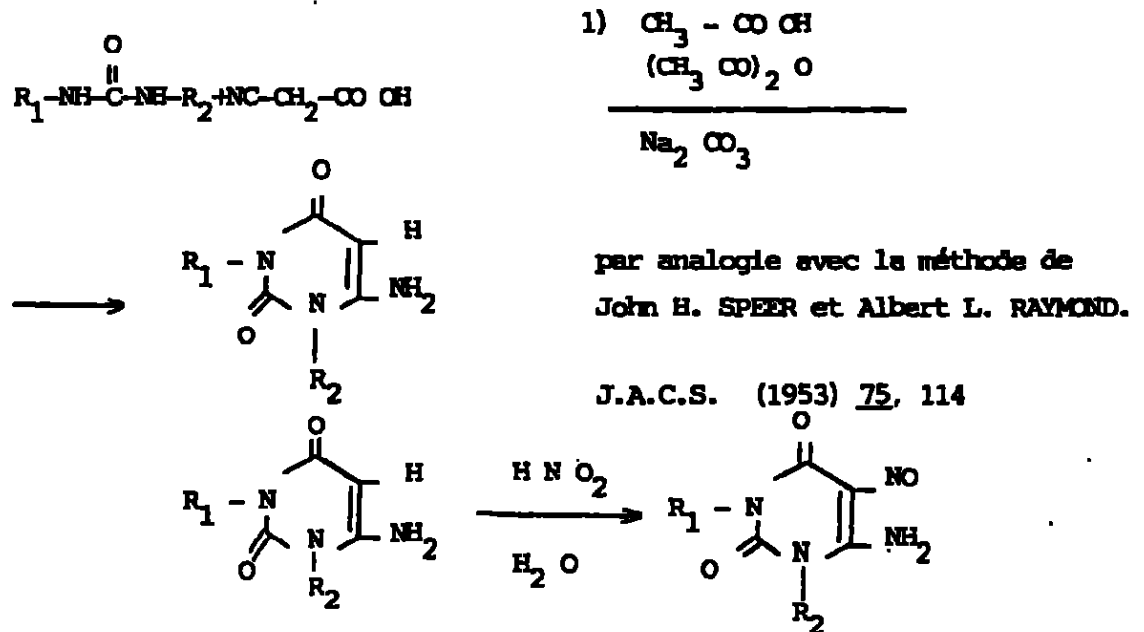
(IX)



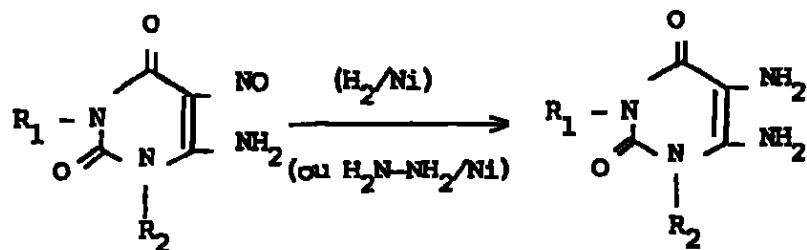
R ₁	R ₂	R ₃	Z	P.F. (Kofler) en °C
CH ₃	CH ₃	H	(CH ₂) ₂	198
CH ₃	CH ₃	H	(CH ₂) ₃	192
CH ₃	CH ₃	CH ₃	(CH ₂) ₂	156
CH ₃	CH ₃	CH ₃	(CH ₂) ₃	96
CH ₃	-CH ₂ -CH-(CH ₃) ₂	H	CH ₂	196
CH ₃	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₂	165
CH ₃	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₃	144
CH ₃	-CH ₂ -CH-(CH ₃) ₂	CH ₃	(CH ₂) ₂	82
CH ₃	-CH ₂ -CH-(CH ₃) ₂	CH ₃	(CH ₂) ₃	76
CH ₃	-CH ₂ -CH=CH ₂	H	(CH ₂) ₃	160
CH ₃	-CH ₂ -CH=CH ₂	CH ₃	(CH ₂) ₃	44-45
CH ₃	-CH ₂ -CH ₂ -CH ₃ *	CH ₃	(CH ₂) ₃	67
C ₂ H ₅	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₃	137-139
C ₂ H ₅	-CH ₂ -CH-(CH ₃) ₂	CH ₃	(CH ₂) ₃	80
CH ₃		H	(CH ₂) ₃	216
CH ₃		CH ₃	(CH ₂) ₃	163
H	-CH ₂ -	H	(CH ₂) ₂	190
H	-CH ₂ -CH-(CH ₃) ₂	H	(CH ₂) ₂	250

* Ce dérivé propyl-3 a été préparé par réduction du dérivé allyl-3 correspondant, sous une pression d'hydrogène de 6 atmosphères en présence de nickel comme catalyseur.

N.B. : Les dérivés de formule VI ont été préparés selon le schéma opératoire ci-après :

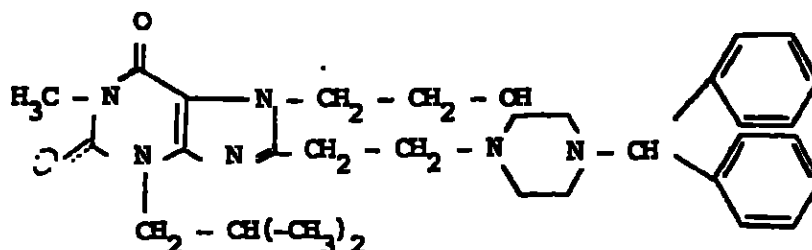


par analogie avec la méthode de G.N. KRUTOVSKIIE et coll.
Pharmaceutical Chemistry Journal (1977) 11(2), 224



EXEMPLE 31 :

Méthyl-1 isobutyl-3 (hydroxy-2 éthyl)-7 [(diphénylméthyl-4 pipérazinyl)-2 éthyl] -8 xanthine



5 10 g de méthyl-1 isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-2 éthyl] -8 xanthine et 37,6 g de carbonate de potassium sont agités dans 200 ml de diméthylformamide et portés à 100°C. On ajoute rapidement 10 ml de chlorhydrate du glycol et agite 1 heure à 110°C. On ajoute
10 ensuite rapidement 20 ml de chlorhydrique du glycol et maintient 1 heure à 110°C sous agitation, on ajoute ensuite 40 g de carbonate de potassium, puis 20 ml de chlorhydrate du glycol. L'ensemble est maintenu à 110°C pendant 3 heures 30, puis à température ambiante pendant
15 72 heures. Le mélange est alors concentré à sec, le résidu est repris dans un mélange de chlorure de méthylène et d'eau. La phase organique est séchée sur sulfate de sodium puis concentrée à sec. On effectue une chromatographie sur 750 g de silice (0,04-0,063 mm). On élue
20 d'abord, au chlorure de méthylène, puis à l'acétate d'éthyle pur, ensuite avec un mélange acétate d'éthyle-méthanol (95-5) et enfin avec un mélange acétate d'éthyle-méthanol (90-10).

On obtient 8,6 g de base que l'on dissous dans 50 ml
25 d'isopropanol. On ajoute suffisamment d'éther chlorhydrique pour avoir un milieu légèrement acide. On précipite le chlorhydrate du produit cherché avec un gros excès d'éther anhydre. On essore et lave à l'éther. Après séchage, à 115°C sous 0,6 mm Hg, on obtient 8,7 g de

dichlorhydrate de méthyl-1 isobutyl-3 (hydroxy-2 éthyl)-7
[(diphénylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, P.F.
(capillaire) 190-200°C (isopropanol/éther) ;
Rendement 71 %.

5 EXEMPLE 32 :

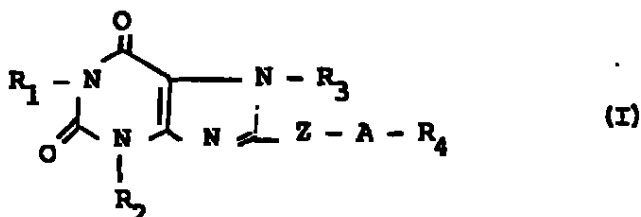
De la même façon a été préparée :

La méthyl-1 isobutyl-3 (dihydroxy-2,3 propyl)-7 [(diphé-
nylméthyl-4 pipérazinyl)-2 éthyl]-8 xanthine, dont le
dichlorhydrate fond (capillaire) à 145-155°C (isopro-
10 panol/éther).

R E V E N D I C A T I O N S

1) Les dérivés de la xanthine de formule générale

I :



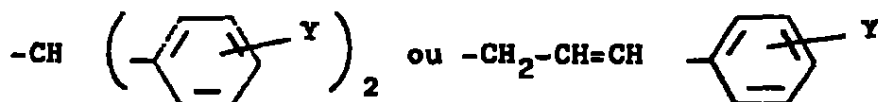
5 dans laquelle :

- R_1 représente un atome d'hydrogène ou un radical alkyle, en chaîne droite ou ramifiée, renfermant de 1 à 5 atomes de carbone ;

- R_2 représente un radical hydrocarboné renfermant 10 jusqu'à 6 atomes de carbone, en chaîne droite ou ramifiée et comportant éventuellement une double liaison, un radical phényle, ou un radical benzyle ;

- R_3 représente un atome d'hydrogène ou un radical alkyle, hydroxyalkyle ou dihydroxyalkyle renfermant 15 chaoun de 1 à 5 atomes de carbone ;

- R_4 représente un groupe de formule :



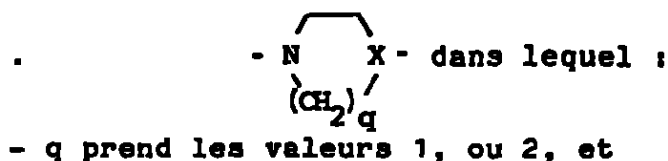
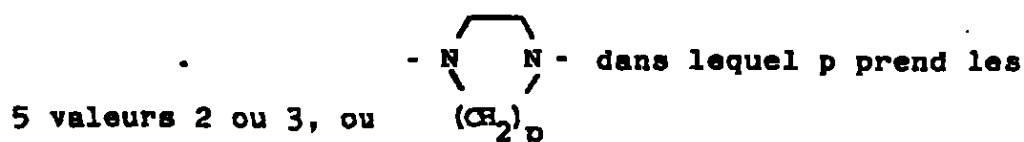
dans lesquelles Y représente un atome d'hydrogène ou d'halogène ou un radical alkyle ou alkoxy renfermant 20 chaoun de 1 à 5 atomes de carbone, ou un radical hydroxyle ;

- Z représente un radical méthylène ou un radical hydrocarboné en chaîne droite ou ramifiée, ayant de 2 à 5

- 23 -

atomes de carbone, éventuellement substitué par un radical hydroxyle, et

- A représente un reste aminé de type :



- X représente une liaison simple, un atome d'oxygène ou un groupe



- 10 dans lequel R_5 représente un atome d'hydrogène ou un radical alkyle ou alkylène ayant chacun de 1 à 5 atomes de carbone ; ou



. m représente un nombre entier de 2 à 6 et

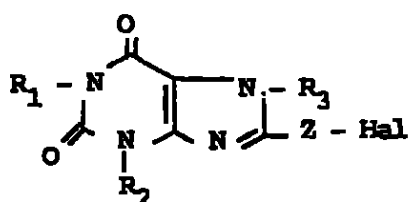
- 15 . R_6 représente un radical alkyle renfermant de 1 à 5 atomes de carbone.

2) Les sels des composés de la revendication 1 avec des acides appropriés.

3) Les sels selon la revendication 2 qui sont physiologiquement tolérables.

4) La méthyl-1 isobutyl-3 [(diphénylméthyl-4 pipérazinyl-2 éthyl]-8 xanthine.

- 5) la méthyl-1 isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine.
- 6) La diméthyl-1,7 isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-3 propyl] -8 xanthine.
- 5 7) La méthyl-1 isobutyl-3 [(diparafluorophényl-méthyl-4 pipérazinyl)-2 éthyl] -8 xanthine.
- 8) La diméthyl-1,7 isobutyl-3 [(diparafluorophényl-méthyl-4 pipérazinyl)-3 propyl] -8 xanthine.
- 9) La méthyl-1 isobutyl-3 [(diphénylméthoxy-4 10 pipéridino)-2 éthyl] -8 xanthine.
- 10) La diméthyl-1,7 isobutyl-3 [(diphénylméthoxy-4 pipéridino)-3 propyl] -8 xanthine.
- 11) La méthyl-1 isobutyl-3 [(diparafluorophényl-méthyl-4 pipérazinyl)-2 éthyl] -8 xanthine.
- 15 12) L'isobutyl-3 [(diphénylméthyl-4 pipérazinyl)-2 éthyl] -8 xanthine.
- 13) La méthyl-1 isobutyl-3 (dihydroxy-2,3 propyl)-7 [(diphénylméthyl-4 pipérazinyl)-2 éthyl] -8 xanthine.
- 14) Le procédé de préparation des composés de la 20 revendication 1, caractérisé en ce que l'on condense un dérivé halogéné de formule générale II :

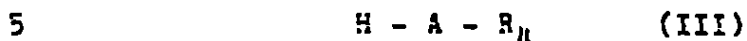


(II)

- 25 -

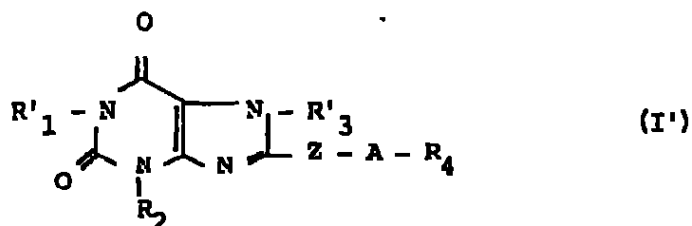
dans laquelle R_1 , R_2 , R_3 et Z ont les significations définies dans la revendication 1, et Hal représente un atome de chlore ou de brome.

avec un dérivé aminé de formule générale III :



dans laquelle A et R_{11} ont les significations énoncées dans la revendication 1.

15) Le procédé de préparation de composés de la revendication 1 répondant à la formule générale I' :



dans laquelle :

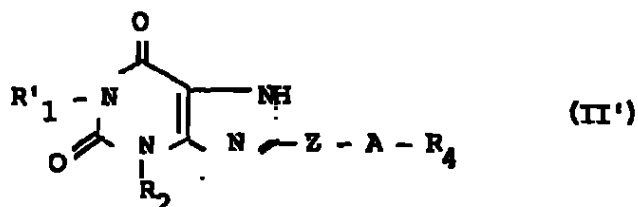
- R_2 , R_4 , Z et A ont les significations définies dans la revendication 1,

- R'_1 représente un radical alkyle en chaîne droite 15 ou ramifiée renfermant de 1 à 5 atomes de carbone, et

- R'_3 représente un radical alkyle, hydroxyalkyle ou dihydroxyalkyle contenant chacun de 1 à 5 atomes de carbone,

- caractérisé en ce que :

l'on condense un dérivé de formule générale II' :



dans laquelle :

- R'_1 , R_2 , R_4 , Z et A sont tels que précédemment
5 définis,

- avec un dérivé halogéné de formule générale
III' :



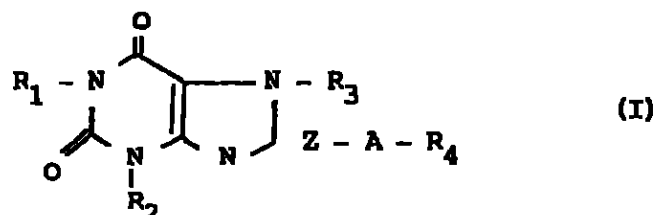
dans laquelle R'_3 a la signification énoncée
10 précédemment et X représente un atome de chlore ou de
brome.

16) Les compositions pharmaceutiques contenant comme
principe actif un composé selon les revendications 1 ou 3
à 13, avec les excipients pharmaceutiques appropriés.

15 17) Les compositions pharmaceutiques selon la reven-
dication 16 présentées sous une forme convenant pour le
traitement de toutes les maladies où il est nécessaire
d'inhiber les réactions antigène-anticorps.

REVENDICATIONS

1) Procédé de préparation des dérivés de la xanthine de formule générale I :



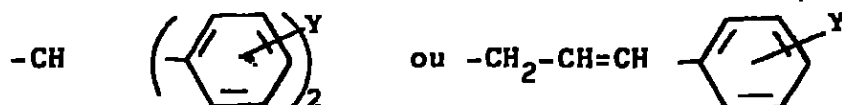
5 dans laquelle :

- R_1 représente un atome d'hydrogène ou un radical alkyle, en chaîne droite ou ramifiée, renfermant de 1 à 5 atomes de carbone ;

- R_2 représente un radical hydrocarboné renfermant 10 jusqu'à 6 atomes de carbone, en chaîne droite ou ramifiée et comportant éventuellement une double liaison, un radical phényle, ou un radical benzyle ;

- R_3 représente un atome d'hydrogène ou un radical alkyle, hydroxyalkyle ou dihydroxyalkyle renfermant 15 chacun de 1 à 5 atomes de carbone ;

- R_4 représente un groupe de formule :



dans lesquelles Y représente un atome d'hydrogène ou d'halogène, un radical alkyle ou alkoxy renfermant chacun 20 de 1 à 5 atomes de carbone, ou un radical hydroxyle ;

- Z représente un radical méthylène ou un radical hydrocarboné en chaîne droite ou ramifiée, ayant de 2 à 5

- 28 -

atomes de carbone, éventuellement substitué par un radical hydroxyle, et

- A représente un reste aminé de type :

5 valeurs 2 ou 3, ou $\begin{array}{c} \diagup \quad \diagdown \\ \text{N} \quad \text{N} \\ \diagdown \quad \diagup \\ (\text{CH}_2)_p \end{array}$ dans lequel p prend les

- q prend les valeurs 1, ou 2, et $\begin{array}{c} \diagup \quad \diagdown \\ \text{N} \quad \text{N} \\ \diagdown \quad \diagup \\ (\text{CH}_2)_q \end{array}$ dans lequel :

- X représente une liaison simple, un atome d'oxygène ou un groupe



10 dans lequel R_5 représente un atome d'hydrogène ou un radical alkyle ou alkylène ayant chacun de 1 à 5 atomes de carbone ; ou

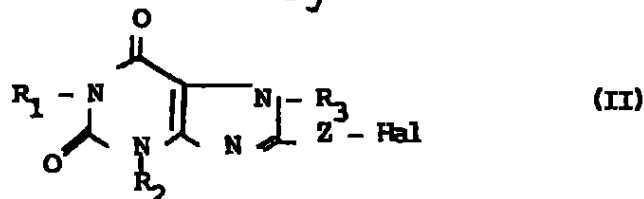
- N - $(\text{CH}_2)_m$ - N dans lequel $\begin{array}{c} \text{N} \\ | \\ \text{R}_6 \end{array}$ $\begin{array}{c} \text{N} \\ | \\ \text{R}_6 \end{array}$ m représente un nombre entier de 2 à 6 et

15 . R_6 représente un radical alkyle renfermant de 1 à 5 atomes de carbone.

- et de leurs sels d'addition avec des acides appropriés,

- caractérisé en ce que l'on condense un dérivé 20 halogéné de formule générale II :

- 29 -



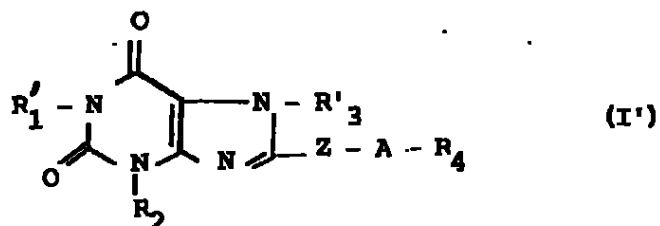
dans laquelle R_1 , R_2 , R_3 et Z ont les significations ci-dessus définies et Hal représente un atome de chlore ou de brome,

5 avec un dérivé aminé de formule générale III :



dans laquelle A et R_4 ont les significations énoncées précédemment ; et si on le désire on traite les dérivés I ainsi obtenus avec des acides appropriés pour 10 donner les sels d'addition correspondants.

2) Le procédé de préparation des dérivés de la xanthine répondant à la formule générale I' :



dans laquelle :

15 - R_2 , R_4 , Z et A ont les significations définies dans la revendication 1,

- $R'1$ représente un radical alkyle en chaîne droite ou ramifiée renfermant de 1 à 5 atomes de carbone, et

- $R'3$ représente un radical alkyle, hydroxyalkyle ou 20 dihydroxyalkyle contenant chacun de 1 à 5 atomes de carbone,

- et de leurs sels d'addition avec des acides appropriés ;

l'on condense un dérivé de formule générale II' :



dans laquelle :

- R'_1, R_2, R_4, Z et A sont tels que précédemment définis.

10 III' : - avec un dérivé halogéné de formule générale



dans laquelle R'₃ a la signification énoncée précédemment et X représente un atome de chlore ou de brome ;

15 - et si on le désire on traite les dérivés I' ainsi obtenus avec des acides appropriés pour donner les sels d'addition correspondants.



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RAPPORT DE RECHERCHE EUROPEENNE

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Numéro de la demande

EP 83 40 1518

DOCUMENTS CONSIDERES COMME PERTINENTS			
Catégorie	Citation du document avec indication, en cas de besoin, des parties pertinentes	Revendication concernée	CLASSEMENT DE LA DEMANDE (Int. Cl. 2)
Y	FR-A-2 489 331 (EISAI) * revendications; tableau 2; page 21, tableau 4; page 24, exemples 1,70-78,97,134-142 *	1-3,14 -17	C 07 D 473/06 C 07 D 473/08 C 07 D 473/10 C 07 D 473/12 A 61 K 31/52 C 07 D 239/54
Y	FR-M- 7 828 (INSTITUTO DE INVESTIGACIONES TERAPEUTICAS) * en entier *	1-3,15 -17	
A	EP-A-0 023 350 (EISAI) * revendications; exemple 32 *	1-3,14 ,16,17	
A	FR-M- 5 086 (CHUGAI SEIYAKU) * résumé *	1-3,14 ,16,17	
A	CH-A- 409 974 (MERCKLE) * revendications; exemple 10 *	1-3,16 ,17	C 07 D 473/00 A 61 K 31/00
P,A	FR-A-2 514 003 (INVESTIGACION TECNICA Y APLICADA) * en entier *	1-3,15 -17	

Le présent rapport de recherche a été établi pour toutes les revendications			
Lieu de la recherche LA HAYE		Date d'achèvement de la recherche 05-11-1983	Examineur NUYTS A.M.K.A.
CATEGORIE DES DOCUMENTS CITES X : particulièrement pertinent à lui seul Y : particulièrement pertinent en combinaison avec un autre document de la même catégorie A : arrière-plan technologique O : divulgation non-écrite P : document intercalaire T : théorie ou principe à la base de l'invention E : document de brevet antérieur, mais publié à la date de dépôt ou après cette date D : cité dans la demande L : cité pour d'autres raisons & : membre de la même famille, document correspondant			

0103497A1.1.1

HELLMUT BREDERECK, EDGAR SIEGEL und BALDUR FÖHLISCH

Synthesen in der Purinreihe, XIII¹⁾

Über die Darstellung einiger Xanthin-aldehyde-(8)

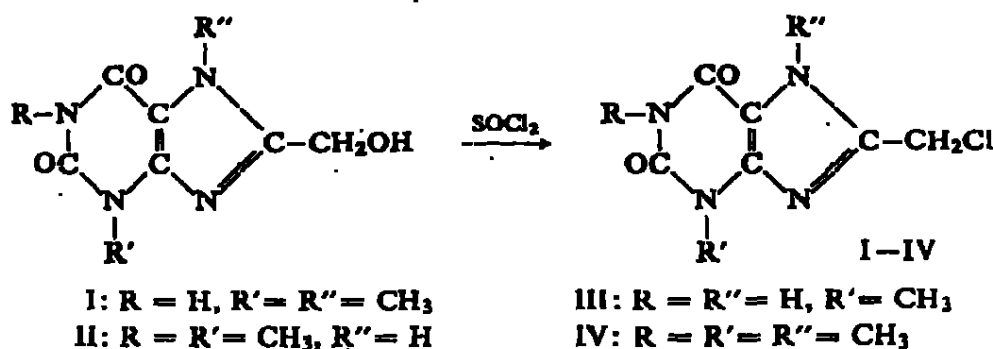
Aus dem Institut für Organische Chemie und Organisch-Chemische Technologie der Technischen Hochschule Stuttgart

(Eingegangen am 26. Juli 1961)

Aus 8-Hydroxymethyl-xanthinen und Thionylchlorid erhält man 8-Chlormethyl-xanthine, deren Pyridiniumsalze sich mit *p*-Nitroso-dimethylanilin in Nitrone überführen lassen. Aus den Hydrochloriden von Theophyllin- und Theobrominaldehyd-(8)-nitrone gewinnt man die entsprechenden 8-Aldehyde. Eine einfache Synthese der Xanthin-aldehyde-(8) fanden wir in der Oxydation von 8-Hydroxymethyl-xanthinen mit Natriumdichromat in Eisessig.

In der Reihe der Purinaldehyde sind bisher Purinaldehyd-(6)²⁾, Coffeinaldehyd-(8)³⁾, 2-Methyl-6-morpholino- und 2-Methyl-6-diäthylamino-purin-aldehyd-(8)⁴⁾ bekannt. Im folgenden beschreiben wir neue Synthesen von Xanthin-aldehyden-(8).

Ausgehend von den entsprechenden 8-Hydroxymethyl-xanthinen haben wir zunächst mit Thionylchlorid die 8-Chlormethyl-Verbindungen des Theobromis (I), Theophyllins (II) und 3-Methyl-xanthins (III) sowie die bereits bekannte 8-Chlormethyl-Verbindung des Coffeins (IV) dargestellt⁵⁾.



Die 8-Hydroxymethyl-xanthine sind leicht über die 4,5-Diamino-uracile durch Umsetzung mit α -Hydroxycarbonsäuren zugänglich⁶⁾. 8-Hydroxymethyl-theobromin haben wir durch partielle Methylierung von 3-Methyl-8-hydroxymethyl-xanthin erhalten. Das bereits bekannte 8-Hydroxymethyl-coffein haben wir in einfacher Weise durch Umsetzung von Coffein mit Paraformaldehyd in Eisessig/HCl dargestellt. Über die Darstellung des 8-Hydroxymethyl-xanthins berichten wir weiter unten.

1) XII. Mitteil.: H. BREDERECK, H. HERLINGER und I. GRAUDUMS, Chem. Ber. 95, 54 [1962].

2) A. GINER-SOROLLA, I. ZIMMERMANN und A. BENDICH, J. Amer. chem. Soc. 81, 2515 [1959].

3) E. S. GOLOVCHINSKAYA, J. gen. Chem. (russ.) 18, 2129 [1948]; C. A. 43, 3794 [1949].

4) R. HULL, J. chem. Soc. [London] 1958, 4069.

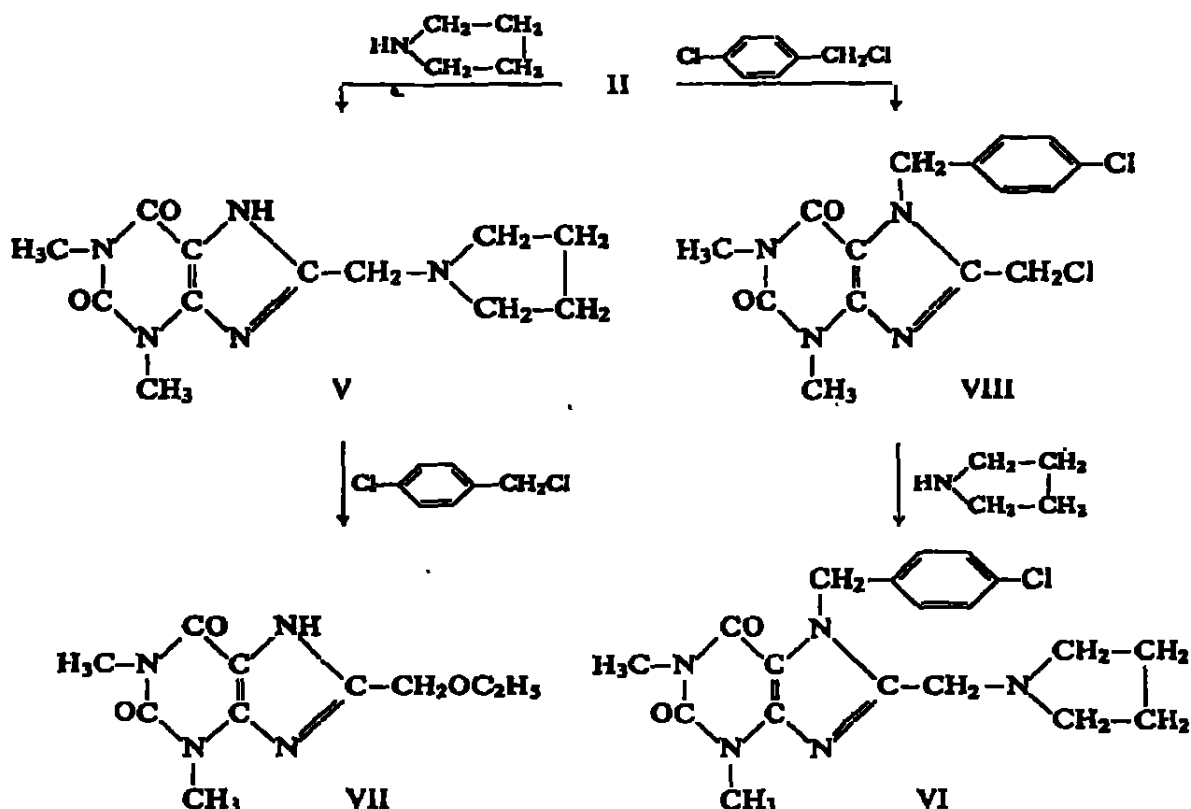
5) E. SIEGEL, Dissertat. Techn. Hochschule Stuttgart 1955.

6) Dtsch. Pat. 212 711 (1900); Fedl. 2 1010 (1900); 10101

228
277

Die 8-Chlormethyl-xanthine haben wir mit verschiedenen Aminen (*N*-Benzyl-anilin, Pyrrolidin) zu den entsprechenden 8-Aminomethyl-xanthinen umgesetzt.

Bei der Umsetzung von II mit Pyrrolidin haben wir das 8-Pyrrolidinylmethyl-theophyllin (V) erhalten, das mit *p*-Chlor-benzylchlorid/Natriumalkoholat in absol. Äthanol jedoch nicht das erwartete 7-[*p*-Chlor-benzyl]-8-pyrrolidinylmethyl-theophyllin (VI) ergab, sondern sich unter Abspaltung des Pyrrolidin-Restes zum 8-Äthoxymethyl-theophyllin (VII) umsetzte. Setzt man jedoch II mit *p*-Chlor-benzylchlorid zum 7-[*p*-Chlor-benzyl]-8-chlormethyl-theophyllin (VIII) um und dieses dann mit Pyrrolidin, so erhält man VI — ein Analogon des Antihistamins Allercur — mit 80% Ausbeute.



Die Beweglichkeit des Chlors in den 8-Chlormethyl-xanthinen nimmt in der Reihenfolge IV $\rightarrow$ I $\rightarrow$ II $\rightarrow$ III zu⁷⁾.

Löst man z. B. die 8-Chlormethyl-Verbindungen bei Raumtemperatur in salpetersaurer Silbernitratlösung, so beginnt sich die zunächst völlig klare Lösung im Falle der in 7-Stellung nicht substituierten Verbindungen II und III nach wenigen Sekunden zu trüben, nach kurzer Zeit ist die Silberchlorid-Fällung quantitativ. Bei I und IV dagegen erfolgt die Silberchlorid-Fällung erst nach Erwärmen.

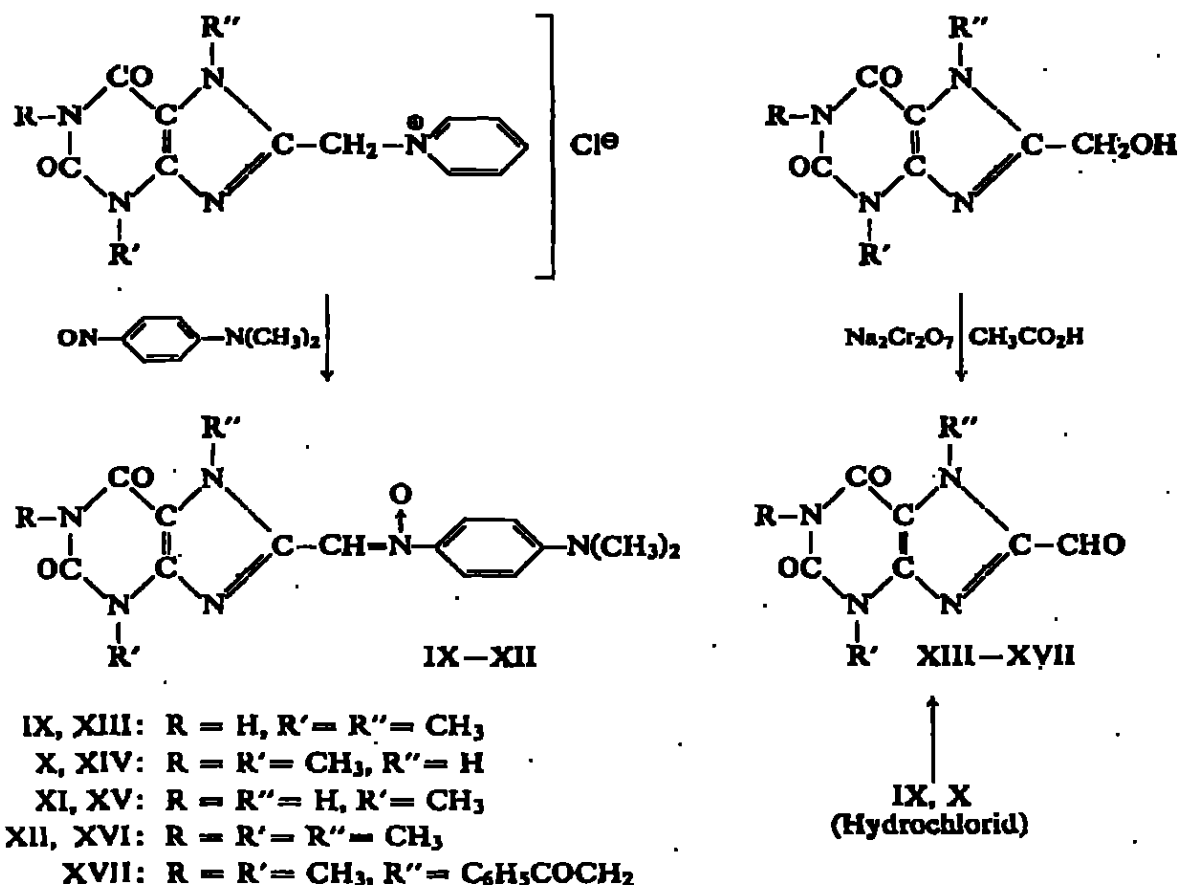
Auch die Umsetzungen mit Pyridin zu den entsprechenden Pyridiniumsalzen zeigen dieselbe Abstufung in der Beweglichkeit des Chloratoms.

II und III bilden mit Pyridin bei Raumtemperatur nach wenigen Sekunden mit ca. 85% Ausb. die Pyridiniumsalze, I erst nach Lösen in Pyridin auf dem siedenden Wasserbad, und IV nach einstündigem Erhitzen in Pyridin (95% bzw. 30% Ausb.). Die 8-Chlormethyl-xanthine I

⁷⁾ H. BREDERECK, Synthesen in der Purin- und Fyrimidinreihe, Festschrift Prof. Dr. A. STOLL, S. 657, Birkhäuser Verlag, Basel 1957.

und II sowie deren Reaktionsprodukte mit verschiedenen Aminen sind parallel zu unseren Untersuchungen zur gleichen Zeit dargestellt und in zwei Patenten beschrieben worden⁸⁾.

Aus den Pyridiniumsalzen haben wir mit *p*-Nitroso-dimethylanilin in alkalischer Lösung nach der Methode von F. KRÖHNKE⁹⁾ die Nitrone IX–XII dargestellt. Nach mehreren vergeblichen Versuchen ist uns die Spaltung von IX zum Theobrominaldehyd-(8) (XIII) über das Hydrochlorid durch kurzes Aufkochen mit Wasser mit 67% Ausb. gelungen. Außerdem konnten wir durch Verreiben des Hydrochlorids von X mit Aceton Theophyllinaldehyd-(8) (XIV) mit geringer Ausbeute (24%) isolieren.



Übersicht über die aus 8-Hydroxymethyl-xanthinen erhaltenen Xanthinaldehyde-(8)

-aldehyd-(8)		Ausb. % d. Th.
Theobromin-	XIII	70
Theophyllin-	XIV	50
3-Methyl-xanthin-	XV	54
Coffein-	XVI	54
7-Phenacyl-theophyllin-	XVII	43

⁸⁾ Amer. Pat. 2879271 [1959]; C. A. 54, 591 [1960]; Dtsch. Bundes-Pat. 1001272 [1957]; C. A. 54, 1569 [1960].

⁹⁾ F. KRÖHNKE, Ber. dtsh. chem. Ges. 71, 2583 [1938]; F. KRÖHNKE und E. Börner, Ber. dtsh. chem. Ges. 69, 2026 [1936].

Viele Verbindungen besitzen keinen exakten Schmp., sie beginnen meist mehrere Grade unter dem angegebenen Schmp. zu sintern. Als Schmp. wird die Temperatur angegeben, bei der eine völlig klare Schmelze vorliegt. Eine Reihe weiterer Verbindungen schmelzen bis 350° nicht.

3-Methyl-8-hydroxymethyl-xanthin: 8.3 g 3-Methyl-4,5-diamino-uracil und 8.5 g Glykolsäure (krist.) werden in der Reibschale innig verrieben und 1 Stde. auf dem siedenden Wasserbad erhitzt. Zu der erstarrten Schmelze gibt man 55 ccm Wasser, neutralisiert mit festem Natriumhydroxyd, gibt 55 ccm 2*n* NaOH zu, kocht die klare Lösung 2 1/2 Stdn. unter Rückfluß, saugt den ausgefallenen Niederschlag ab, säuert die heiße Lösung mit Schwefelsäure auf pH 4 an, saugt den nach Anreiben und Belassen im Kühlschrank ausgefallenen kristallinen Niederschlag ab, wäscht mit Wasser und Äthanol und trocknet bei 110°. Ausb. 5.8 g (50% d. Th.). Nach Umkristallisieren aus der 15fachen Menge Wasser Zers. ab ca. 300° unter schwacher Gasentwicklung und Verkohlung.

$C_7H_8N_4O_3 \cdot H_2O$ (214.2) Ber. C 39.25 H 4.71 N 26.16 H_2O 8.42
Gef. C 39.27 H 4.71 N 26.43 H_2O 8.49

8-Hydroxymethyl-theobromin: 1.3 g 3-Methyl-8-hydroxymethyl-xanthin werden in 3.5 ccm 2*n* NaOH und 8 ccm Wasser gelöst und auf dem Wasserbad auf 40° erwärmt. Nach Zugabe von 0.88 ccm Dimethylsulfat in 5 ccm Methanol läßt man bei 40° bis zum konstanten pH von 7–7.5 2*n* NaOH zutropfen, saugt den Niederschlag nach Aufbewahren im Kühlschrank ab, wäscht mit wenig Wasser und Methanol und trocknet bei 110°. Ausb. 0.85 g (65% d. Th.). Nach Umkristallisieren aus 55facher Menge Wasser Schmp. 297°.

$C_8H_{10}N_4O_3$ (210.2) Ber. C 45.71 H 4.80 N 26.66 Gef. C 44.99 H 4.94 N 27.16

UV-Spektrum	λ_{max} (m μ)	$\log \epsilon_{max}$
in <i>n</i> /10 NaOH	< 220; [236]; 276	— ; [3.82]; 4.10
bei pH 7	< 220; [233]; 276	— ; [3.63]; 4.05
in <i>n</i> /10 HCl	< 220; [234]; 276	— ; [3.65]; 4.06
R_T (B/Ac) 0.39 R_T (P/NH ₃) 0.59	bei 254 m μ Absorption	

8-Hydroxymethyl-coffein: 12 g Coffein, 12 g Paraformaldehyd, 18 ccm Eisessig und 6 ccm konz. Salzsäure werden in einem Bombenrohr 17 Stdn. auf 160–170° erhitzt. Die klare braune Reaktionslösung wird mit starker Natronlauge (ca. 15–20 g Natriumhydroxyd in wenig Wasser gelöst) auf pH 6 gebracht, noch warm in einen Chloroform-Extraktor gegeben, vor dessen Überlauf eine Schicht feiner Glaswolle das Mitreißen auskristallisierter Substanz verhindert, und 10 Stdn. mit 250 ccm Chloroform extrahiert. Man engt den Auszug auf 100 ccm ein, läßt über Nacht im Kühlschrank stehen, saugt den Niederschlag ab, wäscht dreimal mit Chloroform und trocknet bei 110°. Ausb. 5 g (36% d. Th.), Schmp. 223–224°, nach Umkristallisieren aus 40–50 Teilen Äthanol, Schmp. 225°, Lit.³⁾ 224–226°.

8-Chlormethyl-theobromin (I): 0.7 g 8-Hydroxymethyl-theobromin, 3.5 ccm frisch dest. Thionylchlorid und 2 Tropfen Pyridin werden 6 Stdn. unter Rückfluß (Calciumchloridrohr) gekocht, das überschüss. Thionylchlorid i. Vak. abdestilliert, der Rückstand mit wenig absol. Äthanol und dann mit absol. Äther gewaschen und bei 110° getrocknet, Ausb. 0.7 g (90% d. Th.), nach Umkristallisieren aus 15–20 Tln. absol. Dioxan verfilzte Kriställchen, Schmp. 163° (Lit.⁸⁾: 251–253°).

$C_8H_9ClN_4O_2$ (228.7) Ber. C 42.02 H 3.98 N 24.50 Gef. C 41.83 H 3.71 N 24.02

8-Chlormethyl-theophyllin (II): Zu 15.1 g 8-Hydroxymethyl-theophyllin⁶⁾ gibt man unter Wasserabfuhr 45 ccm Thionylchlorid und 2 Tropfen Pyridin.

4 Stdn. unter Rückfluß (Calciumchloridrohr) auf dem siedenden Wasserbad, destilliert das überschüss. Thionylchlorid i. Vak. ab, kocht den Rückstand kurz mit 30 ccm absol. Äthanol auf, saugt ab und trocknet bei 110°. Ausb. 14.9 g (90% d. Th.), rötlich-gelbes Pulver, Schmp. 238–240°, nach Umkristallisieren aus absol. Dioxan und Waschen mit absol. Äthanol farblose Kristalle, Schmp. 241° (Lit.⁸⁾: 249°).

$C_8H_9ClN_4O_2$ (228.7) Ber. C 42.04 H 3.98 Cl 15.50 N 24.50
Gef. C 41.25 H 4.06 Cl 15.42 N 24.22

3-Methyl-8-chlormethyl-xanthin (III): Analog I aus 1 g **3-Methyl-8-hydroxymethyl-xanthin**, 10 ccm **Thionylchlorid** und 2 Tropfen Pyridin. Ausb. 0.92 g (92% d. Th.), nach Umkristallisieren aus 100facher Menge absol. Dioxan und Einengen i. Vak. auf 1/10 des Volumens dünne Prismen, kein Schmp.

$C_7H_7ClN_4O_2$ (214.6) Ber. C 39.18 H 3.29 N 26.11 Gef. C 39.64 H 3.71 N 25.39

8-Pyrrolidinylmethyl-theophyllin (V): Zu der siedenden Suspension von 3.43 g **II** in 150 ccm absol. Äthanol gibt man 2.5 g frisch dest. trockenes **Pyrrolidin**, kocht 15 Stdn. unter Rückfluß, engt die klare bräunlichgelbe Lösung auf ca. 10 ccm ein, läßt im Kühlschrank erkalten, saugt den Niederschlag ab, wäscht mit wenig Äthanol und löst ihn sofort in 25 ccm siedendem Methanol. Nach Absaugen wird das Filtrat auf 3–5 ccm eingengt, nach Abkühlen werden die ausgefallenen feinen Nadelchen abgesaugt, mit Methanol gewaschen und bei 110° getrocknet. Ausb. 1.9 g, Schmp. 203°. Aus der Mutterlauge und nach Umkristallisieren aus Benzol noch 0.3 g, Schmp. 198°, Gesamtausb. 55% d. Th.; die nach Umkristallisieren aus der 50fachen Menge Benzol erhaltenen Nadelchen vom Schmp. 203° werden direkt zu VII umgesetzt. Analysiert wird V als **Silbersalz**: Man gibt die heiße Lösung von 0.2 g Silbernitrat in 0.5 ccm Wasser zur ebenfalls heißen Lösung von 0.26 g V in 1 ccm konz. Ammoniak: 0.35 g (95% d. Th.).

$AgC_{12}H_{16}N_5O_2$ (370.2) Ber. C 38.93 H 4.36 Gef. C 38.57 H 4.40

3-Methyl-8-[N-benzyl-anilinomethyl]-xanthin: 0.21 g **III** und 0.37 g frisch dest. **N-Benzyl-anilin** werden, gut durchmischt, im Ölbad in einem schwachen, trockenen Stickstoffstrom 12 Stdn. auf 150° erhitzt (Steigrohr). Nach Erkalten wird das feste Reaktionsprodukt pulverisiert, dreimal mit je 3 ccm Wasser ausgekocht, heiß abgesaugt, der Rückstand mit Wasser und Äthanol gewaschen und bei 110° getrocknet. Ausb. 0.15 g (40% d. Th.), aus einem Gemisch von 25 ccm Dimethylformamid und 20 ccm Wasser rhombische Plättchen vom Schmp. 250°.

$C_{20}H_{19}N_5O_2$ (361.4) Ber. C 66.48 H 5.26 Gef. C 66.56 H 5.05

8-[N-Benzyl-anilinomethyl]-theophyllin: 0.23 g **II** werden in einer Lösung von 0.37 g frisch dest. **N-Benzyl-anilin** in 10 ccm absol. Äthanol suspendiert und 15 Stdn. unter Rückfluß gekocht. Nach Abkühlen (Kühlschrank) wird abgesaugt, der Rückstand mit Äthanol gewaschen und bei 110° getrocknet, Ausb. 0.31 g (82% d. Th.), aus Äthanol unter Zusatz von wenig Dioxan rhombische Prismen, Schmp. 206°, Lit.⁸⁾: Schmp. 211°.

$C_{21}H_{21}N_5O_2$ (375.4) Ber. C 67.15 H 5.60 N 18.65 Gef. C 66.49 H 5.42 N 18.63

8-[N-Benzyl-anilinomethyl]-coffein: Aus 0.24 g **8-Chlormethyl-coffein (IV)** in 0.37 g **N-Benzyl-anilin** und 10 ccm absol. Äthanol wie vorstehend. Die Reaktionslösung wird auf dem Wasserbad auf ca. 3 ccm eingengt, abgekühlt und angerieben. Nach Stehenlassen im Kühlschrank wird abgesaugt, der Rückstand mit wenig kaltem Äthanol gewaschen und bei 110° getrocknet. Ausb. 0.21 g (54% d. Th.), nach Umkristallisieren aus der 40fachen Menge Wasser/Dioxan Schmp. 156°.

$C_{22}H_{21}N_4O_2$ (389.4) Ber. C 67.85 H 5.91 N 18.00 Gef. C 67.61 H 5.65 N 17.40

8-Äthoxymethyl-theophyllin (VII): Zu der Lösung von 0.53 g *V* in 10 ccm absol. Äthanol gibt man eine Lösung von 46 mg Natrium in 2.5 ccm absol. Äthanol, läßt unter Eiskühlung 0.36 g *p*-Chlor-benzylchlorid in 2 ccm absol. Äthanol zutropfen, 1 Stde. bei Raumtemperatur stehen, kocht 4 Stdn. unter Rückfluß, saugt heiß vom Natriumchlorid ab, engt das Filtrat ein, neutralisiert mit wenigen Tropfen Eisessig (pH 7), saugt das hierbei ausgefallene Reaktionsprodukt ab, wäscht mit Wasser und Äthanol und trocknet bei 110°. Ausb. 0.2 g (42% d. Th.). Aus 100 Teilen Wasser/Äthanol (3:2) und aus 200 Teilen Dioxan/Wasser (3:2) verfilzte Nadelchen. Kein Schmp.

$C_{10}H_{14}N_4O_3$ (238.2) Ber. C 50.42 H 5.92 N 23.52 Gef. C 49.22 H 5.63 N 23.91

7-[*p*-Chlor-benzyl]-8-hydroxymethyl-theophyllin: In die Lösung von 1.07 g Natriumhydroxyd in 15 ccm Wasser trägt man unter Rühren 5.6 g 8-Hydroxymethyl-theophyllin⁶⁾ ein, gibt 135 ccm 96-proz. Äthanol zu, läßt abkühlen und der farblosen Suspension 4.86 g *p*-Chlor-benzylchlorid in 25 ccm 96-proz. Äthanol unter Eiskühlung und starkem Rühren innerhalb von 10 Min. zutropfen, erwärmt langsam auf 20° und kocht unter ständigem Rühren ca. 13 Stdn. bis zur völligen Lösung unter Rückfluß. Man saugt heiß ab, engt das Filtrat auf 30–50 ccm ein, läßt erkalten, saugt den Niederschlag ab, wäscht einige Male mit Wasser, rührt 3 Min. gut mit 10 ccm konz. Ammoniaklösung durch (zur Lösung des Ausgangsproduktes), saugt ab, wäscht mit Wasser und Äthanol und trocknet bei 110°. Ausb. 4.8 g (55% d. Th.), aus 60–70 Teilen Äthanol verfilzte Nadelchen, Schmp. 217°.

$C_{15}H_{15}ClN_4O_3$ (334.7) Ber. N 16.75 Gef. N 16.79

7-[*p*-Chlor-benzyl]-8-chlormethyl-theophyllin (VIII): 4.7 g der vorstehend beschriebenen Verbindung und 13 ccm frisch dest. Thionylchlorid werden 6 Stdn. unter Rückfluß gekocht, das überschüss. Thionylchlorid wird i. Vak. abdestilliert, der Rückstand mit 5 ccm Methanol versetzt und wieder i. Vak. zur Trockne eingeeengt. Der farblose Rückstand wird mit 20 ccm Chloroform (über Phosphorpentoxyd getrocknet) aufgekocht, heiß abgesaugt und das Filtrat in einer flachen Schale eingedunstet. Ausb. 3.3 g (67% d. Th.), aus 30–40 Teilen absol. Äthanol verfilzte Nadelchen, Schmp. 170°.

$C_{15}H_{14}Cl_2N_4O_2$ (354.2) Ber. Cl 20.02 Gef. Cl 20.13

7-[*p*-Chlor-benzyl]-8-pyrrolidinylmethyl-theophyllin (VI) (Hydrochlorid): 2.9 g VIII werden in 100 ccm absol. Äthanol suspendiert, mit 1.3 g reinem trockenem Pyrrolidin versetzt, 15 Stdn. unter Rückfluß gekocht, auf 10 ccm eingeeengt, in den Kühlschrank gestellt, vom ausgefallenen Niederschlag abgesaugt, dieser mit Wasser und mehrere Male mit Äthanol gewaschen und bei 110° getrocknet. Ausb. 2.57 g (81% d. Th.), Schmp. 149°.

Hydrochlorid: Zu 2.4 g VI in 5 ccm Wasser gibt man 2 ccm halbkonz. Salzsäure, schüttelt gut durch, versetzt das erstarrte Hydrochlorid mit 15 ccm Wasser, kocht kurz mit Tierkohle auf und saugt durch eine vorgewärmte Nutsche heiß in eine vorgewärmte Saugflasche ab. Nach langsamem Abkühlenlassen des Filtrats wird der ausgefallene Niederschlag abgesaugt, mit wenig Äthanol gewaschen und bei Raumtemperatur i. Vak.-Exsikkator getrocknet. Ausb. 2.4 g (92% d. Th.), verfilzte, prismatische Nadelchen, Schmp. 227°.

$C_{19}H_{22}ClN_5O_2 \cdot HCl$ (424.3) Ber. N 16.52 Gef. N 16.30

***N*-[Theophyllinyl-(8)-methyl]-pyridiniumchlorid:** 1 g II werden unter Rühren in 15 ccm absol. Pyridin rasch gelöst, nach Stehenlassen bei Raumtemperatur und anschließend im Kühlschrank wird der Niederschlag abgesaugt, mit absol. Äther pyridinfrei gewaschen und bei 110° getrocknet. Ausb. 1.2 g (89% d. Th.), kein definierter Schmp. (Lit.⁸⁾: Schmp. 297°).

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N-[Theobrominyl-(8)-methyl]-pyridiniumchlorid: Aus 0.7 g I und 14 ccm absol. Pyridin nach ca. 20 Min. langer Reaktion auf dem siedenden Wasserbad wie vorstehend. Nach Stehenlassen über Nacht bei Raumtemperatur destilliert man das Pyridin i. Vak. bis auf ca. 1 ccm ab, gibt 5 ccm Äther zu, saugt nach Aufbewahren im Kühlschrank ab, wäscht mit Äther und trocknet bei 110°. Ausb. 0.89 g (94% d. Th.), farblose, kurze Prismen, Schmp. 288–290°. Zur Analyse wird die Verbindung in siedendem 70–80-proz. Äthanol gelöst und so viel reines Äthanol zugegeben, bis in der Hitze Trübung erfolgt, verfilzte Nadelchen.

$C_{13}H_{14}N_3O_2[Cl]$ (307.7) Ber. Cl 11.52 Gef. Cl 11.88

N-(3-Methyl-xanthinyl-(8)-methyl)-pyridiniumchlorid: Aus 0.6 g III und 9 ccm absol. Pyridin, wie bei der Darstellung von *N*-[Theophyllin-(8)-methyl]-pyridiniumchlorid beschrieben. Ausb. 0.7 g (85% d. Th.). 0.6 g werden in einem siedenden Gemisch von 6 ccm Äthanol und 1.5 ccm Wasser gelöst, die heiße Lösung langsam zunächst mit 6 ccm Äthanol versetzt, angerieben, dann zur vollständigen Fällung mit 2 ccm Äthanol versetzt, nach kurzem Stehenlassen abgesaugt, mit Äthanol gewaschen und bei 110° getrocknet. Ausb. 0.45–0.5 g (55–60% d. Th.) farblose, zu Büscheln vereinigte Prismen, kein Schmp.

$C_{12}H_{12}N_3O_2[Cl]$ (293.7) Ber. Cl 12.07 Gef. Cl 12.40

N-(Coffeinyl-(8)-methyl)-pyridiniumchlorid: Aus 1 g 8-Chlormethyl-coffein²⁾ und 20 ccm absol. Pyridin durch kurzes Erhitzen auf dem siedenden Wasserbad. Der Niederschlag wird nach 1 stdg. Stehenlassen bei Raumtemperatur abgesaugt, mit absol. Äther gewaschen und bei 110° getrocknet. Ausb. 0.41 g (31% d. Th.), aus Alkohol Nadelchen, Schmp. 248°.

$C_{14}H_{16}N_5O_2[Cl]$ (321.7) Ber. Cl 11.02 Gef. Cl 10.71

Theobrominaldehyd-(8)-[*p*-dimethylamino-phenylnitron] (IX): Der Lösung von 0.74 g *N*-[Theobrominyl-(8)-methyl]-pyridiniumchlorid in 2 ccm Wasser und 11 ccm Äthanol setzt man nach Erhitzen 0.36 g *p*-Nitroso-dimethylanilin zu, danach 2.42 ccm *n* NaOH. Unter Farbumschlag von Tiefgrün nach Gelborange erstarrt die Lösung. Tags darauf wird abgesaugt, mit Wasser und Äthanol gewaschen und bei 110° getrocknet. Ausb. 0.67 g (82% d. Th.), aus 500 Teilen Äthanol/Wasser (4:1) glänzende rostbraune Prismen, kein Schmp.

$C_{16}H_{18}N_6O_3$ (342.4) Ber. N 24.55 Gef. N 23.69

Theophyllinaldehyd-(8)-[*p*-dimethylamino-phenylnitron] (X): Zu der Lösung von 0.62 g *N*-[Theophyllinyl-(8)-methyl]-pyridiniumchlorid in 2 ccm Wasser und 10 ccm Äthanol gibt man 0.3 g *p*-Nitroso-dimethylanilin, zu der entstandenen tiefgrünen Lösung 1 ccm 2*n* NaOH, läßt 36 Stdn. im Brutschrank bei 40° stehen, danach zur Abkühlung kurze Zeit im Kühlschrank, saugt die ausgefallenen gelben Kriställchen ab, wäscht mit Wasser und Äthanol und trocknet bei 110°. Ausb. 0.37 g (53% d. Th.). Aus 100 Teilen Dimethylformamid goldglänzendes Kristallpulver, Schmp. bei raschem Erhitzen 270° (unter starkem Aufschäumen).

$C_{16}H_{18}N_6O_3$ (342.4) Ber. N 24.55 Gef. N 24.38

3-Methyl-xanthinaldehyd-(8)-[*p*-dimethylamino-phenylnitron] (XI): Aus 0.39 g *N*-(3-Methyl-xanthinyl-(8)-methyl)-pyridiniumchlorid in 1 ccm Wasser und 2 ccm Äthanol durch Zugabe von 0.22 g *p*-Nitroso-dimethylanilin und 1.32 ccm *n* NaOH und 5 tägiges Stehenlassen bei Raumtemperatur, Absaugen des gelben Niederschlags, Waschen mit Wasser und Äthanol sowie Trocknen bei 110°, Ausb. 0.24 g (56% d. Th.). Aus 70 Teilen Dimethylformamid/Wasser (1:1) rötlichbraune, glitzernde Prismen, nicht analysenrein. Schmp. nach raschem Erhitzen bei 255–260° (Zers.).

Coffein-aldehyd-(8)-[*p*-dimethylamino-phenylnitron] (XII): Aus 0.76 g *N*-(Coffeinyl-(8)-methyl)-pyridiniumchlorid in 1 ccm Wasser und 10 ccm Äthanol. Nach Zugabe von 0.39 g

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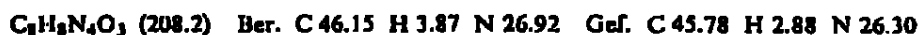
p-Nitroso-dimethylanilin und 1.2 ccm 2 *n* NaOH erfolgt Farbumschlag von Smaragdgrün nach Orangegelb und Erstarren der Lösung. Nach Stehenlassen über Nacht, Waschen mit Wasser und zweimal mit Äthanol sowie Trocknen bei 110° Ausb. 0.1 g (72% d. Th.). Aus 40 Teilen siedendem Äthanol glänzende, gelbe Nadelchen, nach Trocknen i. Vak.-Schrank Schmp. bei langsamem Erhitzen 195° (Zers.).



Theobromin-aldehyd-(8) (XIII)

a) 0.34 g *IX* werden unter Wasserkühlung mit 1 ccm konz. Salzsäure verrieben, das entstandene kristalline Hydrochlorid wird abgesaugt, zweimal mit wenig absol. Äthanol gewaschen, mit 4 ccm Wasser kurz aufgekocht, in Eiswasser abgekühlt, die ausgefallenen farblosen Kristalle abgesaugt, mit Wasser und Äthanol gewaschen und bei 110° getrocknet. Ausb. 0.14 g (68% d. Th.), Schmp. 286°, aus 40 Teilen Dioxan Nadelchen, Schmp. beim raschen Erhitzen 286°.

UV-Spektrum	λ_{max} (m μ)	$\log \epsilon_{\text{max}}$
in <i>n</i> / ₁₀ NaOH	~200; [222]; 279; 348	>4.3; [3.92]; 4.11; 2.82
bei pH 7	~200; [230]; 277; 331	>4.4; [3.91]; 3.97; 3.41
in <i>n</i> / ₁₀ HCl	~200; [230]; 277; 331	>4.38; [3.92]; 3.96; 3.38
R_F (B/Ac) 0.64 R_F (P/NH ₃) 0.40	schwach blaue Fluoreszenz	



b) Der Lösung von 1 g 8-Hydroxymethyl-theobromin in 40 ccm Eisessig läßt man bei 80 bis 85° (Badtemp.) eine Lösung von 0.55 g Natriumdichromat-dihydrat in 25 ccm warmem Eisessig innerhalb 1 Stde. zutropfen, rührt dann noch 30 Min. bei gleicher Temp., destilliert den Eisessig i. Vak. zum größten Teil ab, versetzt den grünen breiigen Rückstand mit 10 ccm Wasser, saugt am andern Morgen ab und wäscht mit Wasser und Methanol. Ausb. 0.69 g (70% d. Th.), Schmp. 286–288° (Zers.), aus Dioxan Schmp. und Misch-Schmp. 288°.

Theophyllin-aldehyd-(8) (XIV)

a) 0.8 g *X* werden mit 2 ccm konz. Salzsäure im Eisbad verrieben, nach ca. 5 Min. erfolgt teilweise Kristallisation des Hydrochlorids; man verrührt mit 15 ccm Aceton im Eisbad, läßt 15 Min. bei 0° stehen, zentrifugiert den Niederschlag ab, wäscht ihn in der Zentrifuge dreimal mit Aceton und je einmal mit Wasser und Aceton und kristallisiert aus Dioxan um. Ausb. 0.11 g (23% d. Th.), kein Schmp., allmählich unscharfe Zersetzung.

b) 20 g 8-Hydroxymethyl-theophyllin werden in 400 ccm Eisessig auf dem siedenden Wasserbad gelöst, man läßt auf 80–85° (Badtemp.) abkühlen, gibt unter Rühren in kleinen Portionen 10 g feingepulvertes Natriumdichromat-dihydrat innerhalb von 15 Min. zu, rührt dann noch 30 Min. bei gleicher Temperatur weiter, läßt über Nacht stehen, saugt ab, wäscht mit Eisessig, dann so lange mit Wasser, bis die Waschflüssigkeit farblos ist, sodann zweimal mit Methanol und trocknet i. Vak.-Exsikkator über Schwefelsäure/Kaliumhydroxyd. Ausb. 11.9 g (60% d. Th.), leicht bräunliche Substanz. Nach zweimaligem Umkristallisieren aus der 60-fachen Menge Dioxan unter A-Kohle-Zusatz 9.8 g (50% d. Th.) gelbstichige Nadelchen, die nach weiterem Umkristallisieren aus Dioxan und A-Kohle farblos sind. R_F -Wert und Analyse sind identisch mit der nach a) dargestellten Verbindung.

UV-Spektrum	$\lambda_{\max}$ (m μ)			log $\epsilon_{\max}$		
in n_{10} NaOH	<220;	241 ;	[259];	333	— 4.08 ;	[3.60]; 4.34
bei pH 7	<200;	240 ;	[259];	335	— 4.05 ;	[3.64]; 4.29
in n_{10} HCl	207;	[230];	274 ;	325	4.39 ;	[3.84]; 3.99 3.38

R_F (B/Ac) Schwanzbildung } schwach blaue Fluoreszenz
 R_F (P/NH₃) 0.55

$C_8H_8N_4O_3$ (208.3) Ber. C 46.15 H 3.87 N 26.92 Gef. C 46.26 H 3.91 N 26.95

3-Methyl-xanthin-aldehyd-(8) (XV): Aus 1.5 g 3-Methyl-8-hydroxymethyl-xanthin in 100 ccm Eisessig und 0.73 g Natriumdichromat-dihydrat in 30 ccm Eisessig bei 80–85°, wie bei XIII beschrieben. Rohausb. 1.1 g (81% d. Th.), braunes Pulver; aus 70facher Menge Wasser unter A-Kohle-Zusatz gelbes Kristallpulver, chromatographisch nicht ganz rein. Zers. über 200°, Ausb. 0.73 g (54% d. Th.).

UV-Spektrum	$\lambda_{\max}$ (m μ)			log $\epsilon_{\max}$		
in n_{10} NaOH	240 ;	278;	347	3.92 ;	3.68;	4.24
bei pH 7	240 ;	261;	331	3.90 ;	3.61;	4.15
in n_{10} HCl	[230];	273;	322	[3.79];	3.99;	3.31

R_F (B/Ac) 0.31 schwach blaue Fluoreszenz

R_F (P/NH₃) 0.37 bei 365 m μ blaue, bei 254 m μ schwach blaue Fluoreszenz

$C_7H_6N_4O_3$ (194.1) Ber. C 43.30 H 3.12 N 28.86 Gef. C 42.51 H 3.70 N 28.37

Coffein-aldehyd-(8) (XVI): Aus 2.24 g 8-Hydroxymethyl-coffein in 50 ccm Eisessig und 1.05 g Natriumdichromat-dihydrat in 40 ccm Eisessig bei 80–85°, wie bei XIII beschrieben. Rohausb. 1.45 g (65% d. Th.), gelbstichige Nadelchen, Schmp. 157–161°, nach Umkristallisieren aus Äthylacetat Ausb. 1.2 g (54% d. Th.), Schmp. 165–169° (Lit.³⁾: 165–167°).

R_F (B/Ac) 0.79

R_F (P/NH₃) 0.86 bei 254 m μ Absorption

Coffein-aldehyd-(8)-anil: Schmp. 225° (Lit.³⁾: 223.5–224°).

7-Phenacyl-8-hydroxymethyl-theophyllin: 4.1 g 8-Hydroxymethyl-theophyllin werden in 10 ccm 2 *n* NaOH unter Erwärmen gelöst. Vom ausgefallenen Natriumsalz wird das Wasser i. Vak. abdestilliert, der Rückstand mit Aceton angerieben, abgesaugt, mit Aceton gewaschen und bei 80° getrocknet. Das Natriumsalz wird in einer Lösung von 4.2 g *o*-Brom-acetophenon in 50 ccm absol. Dimethylformanid suspendiert, 4 Stdn. bei 120–140° gerührt, wobei nach wenigen Min. klare Lösung erfolgt. Das Dimethylformanid wird i. Vak. abdestilliert, der Rückstand mit Wasser verrieben, abgesaugt und gut mit Wasser gewaschen. Rohausb. 6.0 g (93% d. Th.), Schmp. 247° nach vorherigem Sintern; aus 15 Teilen β -Methoxy-Äthanol gelbe, derbe Kristalle, Schmp. 251° nach vorherigem Sintern; Reinausb. 4.8 g (74% d. Th.). Zur Analyse nochmals aus β -Methoxy-Äthanol umkristallisiert.

$C_{16}H_{16}N_4O_4$ (328.3) Ber. C 58.53 H 4.91 N 17.07 Gef. C 58.11 H 5.04 N 16.55

7-Phenacyl-theophyllin-aldehyd-(8) (XVII): Aus 0.98 g 7-Phenacyl-8-hydroxymethyl-theophyllin in 40 ccm Eisessig und 3.2 g Natriumdichromat-dihydrat, wie bei XIV unter b) beschrieben. Rohausb. 0.65 g (66% d. Th.), Schmp. 239° unter Sintern, aus der 40fachen Menge Butanol lockere, farblose Nadeln mit 1 Mol. Kristallbutanol, Schmp. 242°, Sintern ab 218°, Ausb. 0.51 g (43% d. Th.); zur Analyse nochmals aus Butanol umkristallisiert.

$C_{16}H_{14}N_4O_4 \cdot C_4H_9OH$ (400.4) Ber. C 59.99 H 6.04 N 13.99 Gef. C 59.64 H 5.92 N 14.00

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Kristallbutanol wird durch 24stdg. Trocknen bei 120° über P₂O₅ i. Ölpumpenvak. entfernt.
C16H14N4O4 (326.3) Ber. C 58.89 H 4.32 Gef. C 58.31 H 4.27

1.3-Dibenzyl-4-amino-5-[hydroxy-acetamino]-uracil (XVIII)

a) 1.16 g *1.3-Dibenzyl-4.5-diamino-uracil*⁽¹¹⁾ werden mit 0.8 g kristalliner *Glykolsäure* im Mörtel innig verrieben, auf dem Wasserbad 1 Stde. erhitzt, wobei sich zunächst eine tiefrote Schmelze bildet, die sich rasch wieder entfärbt und allmählich erstarrt. Aus 125 ccm Dioxan farblose Nadelchen, Schmp. 243°, Ausb. 1.28 g (67% d. Th.).

C20H20N4O4 (380.4) Ber. C 63.15 H 5.30 N 14.73 Gef. C 62.90 H 5.13 N 14.96

b) 1.0 g *1.3-Dibenzyl-4.5-diamino-uracil*⁽¹¹⁾ werden in einem offenen Erlenmeyer-Kolben mit 2 ccm *Glykolsäure-diäthylester* auf dem Ölbad auf 145–155° erhitzt, abgekühlt und 15 ccm Äther zugegeben. Nach Belassen im Kühlschrank wird der Niederschlag abgesaugt, mit Äther gewaschen und bei 80° getrocknet. Rohausb. 0.84 g (70% d. Th.), Schmp. 216°. Das Rohprodukt kann ohne Reinigung weiter verarbeitet werden.

1.3-Dibenzyl-8-hydroxymethyl-xanthin (XIX): Das innige Gemisch von 16 g *1.3-Dibenzyl-4.5-diamino-uracil* und 10 g kristalliner *Glykolsäure* wird auf dem siedenden Wasserbad 1 Stde. erhitzt, nach Abkühlen die erstarrte Schmelze mit 200 ccm 50-proz. wäßr. Äthanol verrieben und mit festem Natriumhydroxyd gerade alkalisch gemacht. Nach weiterer Zugabe von 7 g Natriumhydroxyd wird 2½ Stdn. unter Rückfluß erhitzt, von der geringen Trübung heiß abgesaugt, das Filtrat mit Eisessig angesäuert, nach Stehenlassen im Kühlschrank der Niederschlag abgesaugt und mehrfach mit 50-proz. Äthanol, dann mit Wasser gewaschen. Rohausb. 13.3 g (73% d. Th.), bräunliche Kristalle, Schmp. 196–202°, nach Umkristallisieren aus Essigester, dann aus Butanol und Aufarbeiten der Mutterlaugen Reinausb. 11.0 g (61% d. Th.), Schmp. 210°, zur Analyse nochmals aus Äthylacetat umkristallisiert.

C20H18N4O3 (362.4) Ber. C 66.28 H 5.01 N 15.46 Gef. C 66.12 H 5.25 N 15.55

8-Hydroxymethyl-xanthin (XX): 4.0 g XIX werden in einem Dreihalskolben mit Rührer und Trockenrohr, gefüllt mit Kaliumhydroxyd, in ca. 150 ccm trockenem, kondensiertem Ammoniak gelöst. Unter Rühren gibt man Natrium in kleinen Stückchen so lange zu, bis die Blaufärbung der Reaktionsmischung mindestens 15 Min. anhält (insgesamt nicht ganz 1.5 g Natrium); danach gibt man etwas festes Ammoniumchlorid zum Abfangen des überschüss. Natriums zu, läßt Ammoniak verdunsten, versetzt den Rückstand mit 50 ccm Wasser, kocht unter Rühren auf, filtriert heiß von der geringen Trübung ab und setzt dem Filtrat in der Siedehitze konz. Salzsäure bis pH 3–4 zu. Nach Stehenlassen in Eis wird das ausgefallene cremefarbene Pulver abgesaugt, mit Wasser, dann mit Methanol gewaschen. Ausb. 1.7 g (83% d. Th.), nach Umfällen mit verd. Natronlauge/HCl in Gegenwart von A-Kohle Ausb. 1.5 g (75% d. Th.), gelbatichige, unter dem Mikroskop rautenförmige Kriställchen, unlöslich in Wasser, Eisessig und Dimethylformamid; beim Erhitzen erfolgt allmähliche Zersetzung. Zur Analyse wird nochmals aus verd. Natronlauge/HCl umgefällt.

UV-Spektrum	λ_{max} (m μ)	$\log \epsilon_{\text{max}}$
in n/10 NaOH	<220; [250]; 286	—; [3.73]; 4.05
R_f (B/Ac) 0.15 R_f (P/NH ₃) 0.17	bei 254 m μ Absorption	

C6H6N4O3 (182.1) Ber. C 39.57 H 3.32 N 30.77 Gef. C 39.98 H 3.39 N 30.74

Murexidreaktion positiv; mit ammoniakalischer Silbernitratlösung erfolgt gallertartige, farblose Fällung.

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2,879,271

BASIC DERIVATIVES OF MONO- AND DI-METHYL XANTHINES, AND A PROCESS OF MAKING SAME

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5 Claims. (Cl. 260-236)

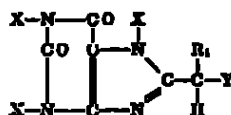
The present invention relates to new and valuable basic derivatives of mono- and di-methyl xanthines, and more particularly to basic derivatives of mono- and di-methyl xanthines having in 8-position a basic side chain, and to a process of making same.

It is one object of the present invention to provide new and valuable basic derivatives of mono- and di-methyl xanthines having in 8-position a basic side chain, said derivatives favorably affecting the circulatory system and causing a decrease in the total peripheral resistance and increase in the peripheral blood flow.

Another object of the present invention is to provide a simple and effective process of producing such basic derivatives of mono- and di-methyl xanthines.

Other objects of this invention and advantageous features thereof will become apparent as the description proceeds.

In principle, the new and valuable basic derivatives of mono- and di-methyl xanthines according to the present invention correspond to the following formula:



In said formula, R_1 indicates hydrogen or a lower alkyl radical, preferably a methyl or an ethyl radical, X indicates hydrogen or methyl groups, not more than two of them being such methyl groups, and Y is a basic nitrogen containing group, such as the primary amino group $-\text{NH}_2$, the secondary amino group $-\text{NHR}$, the tertiary amino group $-\text{NRR}'$, and the quaternary ammonium group $\text{NRR}'\text{R}''(\text{Z})$. In said nitrogen containing group, R, R', and R'' are saturated alkyl radicals with at least two carbon atoms and preferably not more than eight carbon atoms, unsaturated alkyl radicals, alcohol radicals, aryl radicals, preferably phenyl radicals, aralkyl radicals, preferably phenyl alkyl radicals, or cyclohexyl radicals, or, together with the nitrogen atom, a nitrogen containing heterocyclic radical and more particularly a morpholino or pyridinium radical, while Z is an anionic radical, preferably the anionic radical of a mineral acid.

These new compounds and similar compounds can very readily be produced according to the present invention by reacting mono- or di-methyl xanthine-8-carbinols and a halogenating agent, and especially a chlorinating agent such as thionyl chloride or phosphorus chlorides, and by converting the resulting methylated 8-halogeno alkyl xanthine compounds, the halogen atom of which is highly reactive, into the corresponding basic compounds by reaction with ammonia, primary, secondary, or tertiary amines.

The starting materials, the mono- and di-methyl xanthine-8-carbinols, are preferably produced by reacting

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4,5-diamino-2,6-dioxo tetrahydropyrimidines with α -hydroxy carboxylic acids to form the corresponding 5-hydroxy acylo compounds which are then subjected to a cyclization reaction by means of alkaline agents whereby the corresponding xanthine carbinols are obtained. The tetrahydropyrimidine starting materials may be completely or partly methylated. It is, however, also possible to subsequently react the xanthine-8-carbinols with methylating agents.

The new compounds, especially those with a low molecular basic residue in 8-position of the xanthine molecule, are distinguished over known xanthine derivatives by the characteristic feature that they are water-soluble in the form of the free bases. Furthermore, they are readily soluble in alkalies and organic bases as well as in strong inorganic and organic acids, whereby they form water-soluble salts.

It has previously been suggested to substitute theobromine at the nitrogen atom in 1-position by an alkyl or aryl amino alkyl residue. However, the production of the 1-hydroxy alkyl derivatives which are required for said reaction, from theobromine and of the 1-alkyl- or aryl amino-alkyl derivatives from the corresponding 1-halogeno alkyl derivatives, proceeded only with a very low yield.

It has furthermore been suggested to produce 8-chloro methyl xanthine by the action of the calculated amount of elementary chlorine upon 8-methyl xanthine. This process, however, gives satisfactory results only with caffeine. In contrast hereto, chlorination of 8-methyl theobromine with elementary chlorine produced only 8-trichloro methyl theobromine and some unreacted starting material. 8-chloro methyl theophylline cannot at all be produced in this manner. This is not surprising, since theophylline, on reaction with elementary chlorine, is, for the most part, converted into oxidation products.

In contrast hereto, the new compounds and similar compounds can readily be produced by reacting methylated xanthine-8-carbinols which are readily available, with halogenating agents and especially with chlorinating agents such as thionylchloride or phosphorus chlorides. Thereby the corresponding mono-halogeno alkyl-8-xanthines are obtained. Reaction of said mono-halogeno alkyl xanthines with ammonia, primary, or secondary amines produces with an excellent yield the new and similar basic derivatives of mono- and di-methyl xanthines according to the present invention.

The following examples serve to illustrate this invention without, however, limiting the same thereto:

EXAMPLE 1

8-diethylamino methyl theophylline

165 g. of 8-hydroxy methyl theophylline (M.P. 248° C.) are boiled under reflux with 187 g. of thionylchloride (calculated amount) and 250 g. of chloroform for three days. When using a larger excess of thionylchloride it is not necessary to add chloroform as a solvent and diluting agent. After reaction is complete the mixture is allowed to cool, the resulting crystalline mass is filtered off, washed with chloroform and then with water, and recrystallized from glacial acetic acid. In this manner 8-chloro methyl theophylline is obtained in an almost quantitative yield in the form of colorless crystals of the melting point 249° C. Said crystals are insoluble in almost all organic solvents.

19 g. of diethylamine are added at room temperature, while stirring, to a suspension of 30 g. of 8-chloro methyl theophylline in 150 cc. of ethanol. The mixture is heated for one hour on the water bath, whereby the temperature is slowly and gradually increased, until complete solution has taken place. The solution is filtered

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while still hot, cooled, and allowed to crystallize. Crystals of 8-diethylamino methyl theophylline melting, after recrystallization from ethanol, at 176-177° C. are obtained in a yield between 75% and 85% of the theoretical yield.

EXAMPLE 2

8-diethanol amino methyl theophylline

40 g. of 8-chloro methyl theophylline produced according to Example 1, are stirred with 200 cc. of ethanol and 37 g. of diethanol amine in the cold for a certain period of time and the mixture is finally heated until complete solution takes place. The reaction solution is treated with animal charcoal while still hot, filtered, and caused to crystallize by placing the filtrate into a refrigerator. The crystalline mass is filtered off, washed with ethanol, and recrystallized from ethanol. Melting point: 154° C. Yield: 80-90% of the theoretical yield.

EXAMPLE 3

8-di-n-butylamino methyl theophylline

40 g. of 8-chloro methyl theophylline obtained according to Example 1 are placed in a round-bottom flask provided with stirrer, reflux condenser, and dropping funnel and are suspended in 300 cc. of benzene. 45 g. of di-n-butylamine are slowly and gradually added to said chloro compound and benzene, while stirring, whereby increase in temperature of the mixture takes place. Stirring is continued and the mixture is finally heated whereby solution takes place. On allowing said mixture to stand for some time while cooling, the contents of the flask solidify. The resulting crystalline mass is filtered off after standing for some time in a refrigerator, washed with water to dissolve admixed di-n-butylamine hydrochloride, and the resulting 8-di-n-butyl amino methyl theophylline which is difficultly soluble in water, is recrystallized from ethanol. Melting point of the base: 148° C., melting point of the chlorohydrate, after recrystallization from acetone: 200° C. Yield: 90% of the theoretical yield.

The hydrochloride of said 8-di-n-butylamino methyl theophylline is obtained, for instance, by dissolving the base in absolute ethanol and passing gaseous hydrochloric acid into said solution.

EXAMPLE 4

8-morpholino methyl theophylline

23 g. of morpholine are added portion by portion to a suspension of 30 g. of 8-chloro methyl theophylline obtained according to Example 1 in 200 cc. of ethanol. The mixture is stirred for one hour in the cold and finally heated on the water bath until complete solution takes place. Animal charcoal is added to the hot solution which is then filtered and cooled to 0° C. The resulting crystalline mass is washed with a small amount of ethanol and is recrystallized from ethanol. Melting point: 244° C. Yield: about 85% of the theoretical yield.

EXAMPLE 5

8-N-benzyl-N-methylamino methyl theophylline

A solution of 20.5 g. of benzyl methylamine in 200 cc. of ethanol are added to 30 g. of 8-chloro methyl theophylline obtained according to Example 1. The mixture is stirred in the cold for one hour and is then boiled under reflux until the chloro methyl theophylline is completely dissolved. After evaporating the solvent, the residue is treated with water to remove benzyl methylamine chlorohydrate. The remaining crystalline product is recrystallized from ethanol. Melting point: 186° C. Yield: exceeding 80% of the theoretical yield.

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EXAMPLE 6

8-N-β-hydroxy ethyl-N-methylamino methyl theophylline

A suspension of 30 g. of 8-chloro methyl theophylline in 150 cc. of ethanol is reacted with 19.5 g. of β-hydroxy ethyl methylamine as described in Example 5. The resulting crystalline reaction product melts at 160° C. after recrystallization from ethanol. Yield: 75% of the theoretical yield.

EXAMPLE 7

8-diallylamino methyl theophylline

A suspension of 30 g. of 8-chloro methyl theophylline in 150 cc. of ethanol is reacted with 25.5 g. of diallylamine as described in Example 5. The resulting crystalline product, on recrystallization from absolute ethanol, melts at 166° C. Yield: 70% of the theoretical yield.

EXAMPLE 8

8-N-cyclohexyl-N-methylamino methyl theophylline

A suspension of 30 g. of 8-chloro methyl theophylline in 250 cc. of ethanol are reacted with 50 g. of cyclohexyl methylamine as described in Example 5. The reaction mixture is concentrated by evaporation to a volume of 200 cc. whereby a crystalline product is obtained which is filtered off, washed with ethanol, and recrystallized from dilute ethanol. Melting point: 188° C. Yield: 85% of the theoretical yield.

EXAMPLE 9

8-N-isobutyl-N-methylamino methyl theophylline

A suspension of 30 g. of 8-chloro methyl theophylline in 200 cc. of ethanol is reacted with 25 g. of isobutyl methylamine in a similar manner as described in Example 5. The resulting product is recrystallized from ethanol and melts at 159° C. Yield: About 75% of the theoretical yield.

EXAMPLE 10

8-N-[6-methyl heptane-(5')-yl-(2')]N-methylamino methyl theophylline

37 g. of [6-methyl heptane-(5')-yl-(2')] methylamine are added to a suspension of 30 g. of 8-chloro methyl theophylline in 200 cc. of ethanol. The mixture is stirred in the cold for one hour and thereafter for two further hours while heating, finally to boiling temperature. The clear, hot solution is filtered after addition of charcoal, the filtrate is cooled, and the resulting crystalline mass, after standing in the refrigerator, is filtered off and twice recrystallized from ethanol. Melting point: 127-128° C. Yield: 70% of the theoretical yield.

EXAMPLE 11

8-N-phenyl-N-ethylamino methyl theophylline

A suspension of 30 g. of 8-chloro methyl theophylline in 30 cc. of ethanol is boiled with 35 g. of ethyl aniline under reflux for two days. After cooling, the resulting crystalline mass is filtered off, washed with ethanol and acetone, and recrystallized from ethanol containing hydrochloric acid. The chlorohydrate of 8-N-phenyl-N-ethylamino methyl theophylline is obtained in a yield of 80% of the theoretical yield. The melting point is about 194° C. By repeated recrystallization of said chlorohydrate from dilute ethanol, it is hydrolyzed and the free base of the melting point 219° C. is obtained.

EXAMPLE 12

8-N-benzyl-N-cyclohexylamino methyl theophylline

A suspension of 30 g. of 8-chloro methyl theophylline in 200 cc. of ethanol is reacted with 25 g. of benzyl cyclohexylamine as described in Example 5. 8-N-benzyl-N-cyclohexylamino methyl theophylline melting at 175° C. is obtained thereby in a yield of 80% of the theoretical yield.

EXAMPLE 13

8-N-phenyl-N-benzylamino methyl theophylline

50 g. of dry and finely pulverized potassium carbonate and 25 g. of benzyl aniline are added to a suspension of 30 g. of 8-chloro methyl theophylline in 300 cc. of ethanol. The reaction mixture is boiled under reflux for two days while stirring vigorously, filtered while still hot, and cooled to 0° C. Crystalline 8-N-phenyl-N-benzylamino methyl theophylline precipitates. It has a melting point of 211° C. after recrystallization from dilute ethanol. Yield: 70% of the theoretical yield.

EXAMPLE 14

8-cyclohexylamino methyl theophylline

30 g. of 8-chloro methyl theophylline are added in small portions to a solution of 52 g. of cyclohexylamine in 400 cc. of ethanol in the course of three hours while stirring vigorously. Stirring is continued for four hours in the cold and for three more hours while heating the reaction mixture to boiling. The solvent is then evaporated, the residue is boiled with water to remove the di-theophylline compound which is insoluble in water. Said bis-(8,8'-theophylline methyl) cyclohexylamine is recrystallized from glacial acetic acid. Its melting point is 273-275° C.

The aqueous solution obtained on removing said di-theophylline compound is filtered with charcoal; evaporated to dryness in a vacuum, and the residue is recrystallized from ethanol, yielding 8-cyclohexylamino methyl theophylline of the melting point 179-180° C.

EXAMPLE 15

8-isobutylamino methyl theophylline

30 g. of 8-chloro methyl theophylline are added in small portions to a solution of 40 g. of isobutylamine in 400 cc. of ethanol. The reaction mixture is stirred for five hours in the cold and for three more hours while heating. The solvent is evaporated and the resulting residue is boiled with water. The undissolved product is recrystallized from glacial acetic acid, yielding bis-(8,8'-theophylline methyl) isobutylamine of the melting point 289° C.

The aqueous solution obtained thereby is evaporated to dryness and the residue is recrystallized from ethanol, yielding 8-isobutylamino methyl theophylline melting at 203° C.

EXAMPLE 16

Theophylline-8-methyl pyridinium chloride

7 g. of pyridine are added to a suspension of 20 g. of 8-chloro methyl theophylline in 300 cc. of ethanol. The reaction mixture is slowly heated while stirring and is then boiled until complete solution takes place. On cooling, the chloride of the quaternary theophylline-8-methyl pyridinium base precipitates. Its melting point, after recrystallization from ethanol, is 297° C.

EXAMPLE 17

8-*diethylamino ethyl* theophylline

800 g. of thionylchloride are slowly added drop by drop to 110 g. of dry 8-*hydroxy ethyl* theophylline, melting at 225-226° C. Reaction sets in instantaneously but ceases gradually. To complete reaction, the mixture is heated under reflux for five hours. Thereby first a clear solution is obtained whereafter the chloro compound formed slowly crystallizes. After cooling and standing in a refrigerator, the crystals are filtered off and are washed with chloroform and water. The thionylchloride-chloroform solution is subjected to distillation. The residue is treated with water and is combined with the main portion of the crystalline material obtained after washing with chloroform and water. The combined crystals are recrystallized from glacial acetic acid. The resulting 8-*chloro*

ethyl theophylline has a melting point of 318° C. The yield is almost quantitative.

18 g. of diethylamine are added to a suspension of 30 g. of said 8-*chloro ethyl* theophylline in 200 cc. of ethanol. The reaction mixture is stirred at room temperature for one hour, is slowly heated, and finally boiled under reflux until a clear solution results. Charcoal is added to said solution which is then filtered while still hot. The filtrate is allowed to crystallize in a refrigerator. Crystals of 8-*diethylamino ethyl* theophylline of the melting point 186° C., after recrystallization from ethanol, are obtained. The yield is 80% of the theoretical yield.

EXAMPLE 18

8-*(di-n-butylamino ethyl)* theophylline

106.3 g. of di-n-butyl amine are slowly added drop by drop to a solution of 100 g. of 8-*chloro ethyl* theophylline in 400 cc. of benzene. The further procedure is as described in Example 17. Di-butylamine chlorohydrate is dissolved from the reaction mixture by means of water. The resulting crystals of 8-*(di-n-butylamino ethyl)* theophylline are recrystallized from ethanol and melt at 158° C. Yield: 88%.

EXAMPLE 19

8-*(piperidino ethyl)*-3-methyl xanthine

100 g. of 8-*(α -hydroxy ethyl)*-3-methyl xanthine of the melting point 305° C. are added in portions to 250 g. of phosphorus oxychloride. The mixture is heated until complete solution takes place. Thereafter most of the excess phosphorus oxychloride is distilled off in a low vacuum, chloroform is added to the residue, and the mixture is allowed to crystallize on standing in a refrigerator. The crystalline mass is filtered off, washed with water, and boiled with acetone. Melting point: 291° C. Yield: 90%.

21 g. of piperidine are added to a suspension of 30 g. of said 8-*(α -chloro ethyl)*-3-methyl xanthine obtained as described above, in 300 cc. of ethanol. The mixture is stirred at room temperature for two hours and thereafter one more hour at 50-60° C. Finally the mixture is boiled under reflux for four hours. A crystalline mass is obtained on cooling which is filtered off. On recrystallization from dilute ethanol, 8-*(piperidino ethyl)*-3-methyl xanthine of the melting point 235° C. is obtained in a yield of 80%.

EXAMPLE 20

8-*(N-benzyl-N-methylamino ethyl)*-3-methyl xanthine

A solution of 30 g. of 8-*(α -chloro ethyl)*-3-methyl xanthine in 300 cc. of ethanol are reacted with 31.5 g. of benzyl methylamine as described in Example 19. Crystalline 8-*(N-benzyl-N-methylamino ethyl)*-3-methyl xanthine is obtained in a yield of 68%. Melting point, after recrystallization from ethanol: 212° C.

EXAMPLE 21

8-*piperidino methyl* theobromine

500 cc. of thionylchloride are added drop by drop to 250 g. of dry 8-*hydroxy methyl* theobromine melting at 297-299° C. Instantaneously vigorous reaction sets in. Said reaction gradually ceases and the reaction mixture is then boiled under reflux for eight hours. Thereafter the reaction mixture is diluted with chloroform, the mixture is allowed to stand for some time in a refrigerator and the crystalline product is filtered off, washed with chloroform, and then with water. On recrystallization from glacial acetic acid, 8-*chloro methyl* theobromine of the melting point 251-253° C. is obtained. Yield: exceeding 90% of the theoretical yield.

22.5 g. of piperidine are added to a solution of 30 g. of said chloro compound in 300 cc. of ethanol. The reaction is carried out as described in the preceding ex-

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amides. The resulting reaction product, 8-piperidino methyl theobromine, is recrystallized from aqueous ethanol (1:1) and has a melting point of 248-250° C.

EXAMPLE 22

8-di-n-butylamino methyl theobromine

34 g. of di-n-butylamine are reacted with 30 g. of 8-chloro methyl theobromine in 300 cc. of ethanol as described in Example 21. Crystalline 8-di-n-butyl amino methyl theobromine is obtained thereby which melts, after recrystallization from ethanol, at 160° C. Yield: 87% of the theoretical yield.

EXAMPLE 23

8-di-(p-hydroxy ethyl) amino methyl theobromine

27.5 g. of di-ethanolamine are reacted with 30 g. of 8-chloro methyl theobromine in 300 cc. of ethanol in a similar manner as described in Example 21. Crystalline 8-di-(p-hydroxy ethyl) amino methyl theobromine which, after recrystallization from ethanol, melts at 169° C. is obtained in a yield of 79% of the theoretical yield.

EXAMPLE 24

8-diallylamino methyl theobromine

25.5 g. of diallylamine are added to a suspension of 30 g. of 8-chloro methyl theobromine in 200 cc. of ethanol. The reaction is carried out in a similar manner as described in Example 21. Crystalline 8-diallylamino methyl theobromine is obtained in a yield of 89% of the theoretical yield. The melting point of said compound, after recrystallization from dilute ethanol, is at 143-144° C.

EXAMPLE 25

8-N-benzyl-N-methylamino methyl theobromine

32 g. of benzyl methylamine are reacted with a suspension of 30 g. of 8-chloro methyl theobromine in 300 cc. of ethanol in an analogous manner as described in Example 21. Crystalline 8-N-benzyl-N-methylamino methyl theobromine is obtained in a yield of 83% of the theoretical yield. Its melting point, after recrystallization from dioxane, is 239-240° C.

EXAMPLE 26

8-N-(6'-methyl heptene(5')-yl-(2'))-N-methylamino methyl theobromine

A suspension of 30 g. of 8-chloro methyl theobromine in 200 cc. of ethanol is reacted with 37 g. of 6-methyl-amino-2-methyl heptene(2) as described in Example 24. The resulting reaction solution is concentrated by evaporation to a volume of 150 cc. Said solution is kept for a prolonged period of time at 0° C. whereby crystalline 8-N-(6'-methyl heptene(5')-yl-(2'))-N-methylamino methyl theobromine is obtained. Said product is recrystallized from ethanol and melts at 112° C. Yield: 80% of the theoretical yield.

EXAMPLE 27

8-N-phenyl-N-ethylamino methyl theobromine

30.5 g. of ethyl aniline are added to a suspension of 26 g. of 8-chloro methyl theobromine in 200 cc. of ethanol. The mixture is boiled under reflux for five hours while stirring. After cooling, the crystalline product is boiled with dilute ethanol and subsequently with aqueous acetone and is recrystallized from dioxane. Melting point: 230° C. Yield: 84% of the theoretical yield.

The above mentioned compounds and other compounds of similar constitution can be prepared by other suitable methods. For instance, 1,3-dimethyl-4, 5-diamino-2,6-dioxo tetrahydropyrimidine is reacted with α -chloro carboxylic acids or α -chloro carboxylic acid chlorides to 1,3-dimethyl-2,6-dioxo-4-amino 5- α -chloro acyl-amino tetrahydropyrimidine compounds. Reacting

said compounds with amines yields the corresponding 5- α -amino acyl compounds which can be converted into 8- α -amino alkyl methyl xanthines by cyclization by means of alkalies.

Another method of making such new 8- α -amino alkyl methyl xanthines consists in hydrogenating 8-cyano theophylline or theobromine and partly or completely alkylating the resulting 8-amino methyl xanthines.

Still another method of making these compounds comprises subjecting 8-formyl methyl xanthines to a reducing condensation reaction with primary amines. The resulting secondary amines are then alkylated to tertiary or quaternary amines.

The following examples serve to illustrate these other methods of producing said new basic derivatives of mono- and di-methyl xanthines having in 8-position a basic side chain:

EXAMPLE 28

1,3-dimethyl-2,6-dioxo-4-amino-5-diethylamino acetylamino tetrahydropyrimidine

30 g. of 1,3-dimethyl-2,6-dioxo-4-amino-5-chloro acetylamino tetrahydropyrimidine are suspended in a flask provided with a stirrer, reflux condenser, and dropping funnel, in 250 cc. of chloroform. 50 g. of finely pulverized potassium carbonate are also suspended therein. To said suspension, 18 g. of diethylamine are added, the mixture is stirred, in the beginning, at room temperature, and, thereafter, for five hours while boiling on the water bath. The inorganic salts are filtered off, and chloroform and excess of diethylamine are completely distilled off from the resulting filtrate. The residue is recrystallized from acetone. Yield: 65% of the theoretical yield. Melting point: 133° C.

8-diethylamino methyl theophylline

10 g. of said 1,3-dimethyl-2,6-dioxo-4-amino-5-diethylamino acetylamino tetrahydropyrimidine are dissolved in 50 cc. of water. Twice the calculated amount of sodium hydroxide is added thereto, and the mixture is boiled for about three hours. The reaction product is acidified by addition of glacial acetic acid, again rendered alkaline by addition of sodium carbonate, and 8-diethylamino methyl theophylline is extracted with chloroform. After evaporating the chloroform, the residue is recrystallized from ethanol. Yield: 55% of the theoretical yield. Melting point: 176° C.

EXAMPLE 29

1,3-dimethyl-2,6-dioxo-4-amino-5-di-n-butylamino acetylamino tetrahydropyrimidine

50 g. of 1,3-dimethyl-2,6-dioxo-4-amino-5-chloro acetylamino tetrahydropyrimidine are suspended in 200 cc. of ethanol. 52.3 g. of di-n-butylamine are added thereto and the mixture is boiled under reflux for four hours. Clear solution takes place thereby. The mixture is placed into a refrigerator. The precipitated crystals are filtered off and are recrystallized from ethanol. Yield: 85% of the theoretical yield. Melting point: 157-158° C.

8-(di-n-butylamino) methyl theophylline

36.0 g. of said 1,3-dimethyl-2,6-dioxo-4-amino-5-diethylamino acetylamino tetrahydropyrimidine are boiled under reflux in 100 cc. of water containing 9 g. of sodium hydroxide for two hours. The sodium compound of the resulting theophylline derivative crystallizes almost quantitatively on cooling. It is dissolved in water, decomposed by addition of acid, and the base is liberated from the resulting salt with acid by means of sodium carbonate. The precipitated crystals are filtered off, dried, and recrystallized from ethanol. Yield: 91% of the theoretical yield. Melting point: 146-148° C.

The chlorohydrate of said 8-(di-n-butylamino) methyl theophylline, on recrystallization from acetone, has a

melting point of 200° C., its phosphate, on recrystallization from ethanol, has a melting point of 178° C.

EXAMPLE 30

1,3-dimethyl-2,6-dioxo-4-amino-5-morpholino acetylamino tetrahydropyrimidine

23 g. of morpholine are added to 30 g. of 1,3-dimethyl-2,6-dioxo-4-amino-5-chloro acetylamino tetrahydropyrimidine in 200 cc. of ethanol. The mixture is first moderately heated and then boiled under reflux for four hours. The reaction product precipitates in crystalline form on cooling the clear reaction solution. It is filtered off and recrystallized from ethanol. Yield: 88% of the theoretical yield. Melting point: 264-265° C.

8-morpholino methyl theophylline

20 g. of 1,3-dimethyl-2,6-dioxo-4-amino-5-morpholino acetylamino tetrahydropyrimidine are boiled under reflux in about 80 cc. of water with the calculated amount of sodium hydroxide solution for two hours. Carbon dioxide is introduced into the clear solution until all the sodium hydroxide is converted into sodium carbonate. The precipitated crystals are filtered off and recrystallized from dilute ethanol. Yield of 8-morpholino methyl theophylline: 86% of the theoretical yield. Melting point: 243-244° C.

EXAMPLE 31

1,3-dimethyl-2,6-dioxo-4-amino-5-cyclohexyl methylamino acetylamino tetrahydropyrimidine

27.5 g. of cyclohexyl methylamine are added to 30 g. of 1,3-dimethyl-2,6-dioxo-4-amino-5-chloro acetylamino tetrahydropyrimidine in 200 cc. of ethanol and the mixture is boiled under reflux for four hours. The reaction product, on cooling the clear solution, precipitates in the crystalline state. It is filtered off and recrystallized from ethanol. Yield: 82% of the theoretical yield. Melting point: 171-173° C.

8-(cyclohexyl methylamino)-methyl theophylline

20 g. of said 1,3-dimethyl-2,6-dioxo-4-amino-5-cyclohexyl methylamino acetylamino tetrahydropyrimidine are boiled under reflux in 100 cc. of water with twice the calculated amount of sodium hydroxide for two hours. The reaction solution is acidified and 8-(cyclohexyl methylamino)-methyl theophylline is set free from said acid solution by the addition of sodium carbonate. The crystals are filtered off by suction until substantially dry and are recrystallized from ethanol. Yield: 93% of the theoretical yield. Melting point: 203-204° C.

EXAMPLE 32

1,3-dimethyl-2,6-dioxo-4-amino-5-benzyl methylamino acetylamino tetrahydropyrimidine

29 g. of benzyl methylamine are added to 30 g. of 1,3-dimethyl-2,6-dioxo-4-amino-5-chloro acetylamino tetrahydropyrimidine in 150 cc. of ethanol and the mixture is boiled under reflux for four hours. The reaction product precipitates in the crystalline state from the clear solution on cooling. It is filtered off and recrystallized from ethanol. Yield: 75% of the theoretical yield. Melting point: 203-204° C.

8-(benzyl methylamino) methyl theophylline

An excess of 50% over the calculated amount of sodium hydroxide is added to 20 g. of 1,3-dimethyl-2,6-dioxo-4-amino-5-benzyl methylamino acetylamino tetrahydropyrimidine in 100 cc. of water and the mixture is boiled under reflux for two hours. The reaction product is acidified and the base is set free from the resulting acid solution by the addition of sodium carbonate. 8-(benzyl methylamino) methyl theophylline is filtered off and recrystallized from ethanol. Yield: 95% of the theoretical yield. Melting point: 186° C.

The starting material used in the Examples 28 to 32

is prepared by boiling 1,3-dimethyl-2,6-dioxo-4,5-di-amino tetrahydropyrimidine with chloro acetic acid according to the procedure disclosed in German Patent No. 213,711. It was found, however, that the following procedure produces higher yields.

68 g. of chloro acetylchloride are rapidly added drop by drop at room temperature, while stirring, to a suspension of 85 g. of 1,3-dimethyl-2,6-dioxo-4,5-diamino tetrahydropyrimidine in 1200 cc. of chloroform. Thereby the temperature of the reaction mixture gradually increases. The mixture is then heated under reflux on the water bath for 10 hours while stirring. After cooling, the reaction product is separated from the solvent, washed with chloroform, dried in a vacuum, and recrystallized from water. Its melting point is 215-217° C. The yield amounts to 78% of the theoretical yield. The compound is readily soluble in hot water and methanol and insoluble in ether, acetone, and chloroform.

When using α -chloro propionic acid chloride or α -chloro butyric acid chloride in this reaction in place of chloro acetic acid chloride, the corresponding 1,3-dimethyl-2,6-dioxo-4-amino-5-(α -chloro propionyl)- or (α -chloro butyryl) amino tetrahydropyrimidine compounds are obtained which, with tertiary bases, such as diethylamine, di-n-butylamine, morpholine, benzyl methylamine and others and subsequent ring closure, yield the corresponding 8-(diethylamino) ethyl or propyl theophylline, 8-(di-n-butylamino) ethyl or propyl theophylline, etc.

As stated hereinbefore and shown in the examples, the new compounds are capable of forming acid addition compounds with inorganic acids, such as sulfuric acid, hydrochloric acid, hydrobromic acid, phosphoric acid and others, as well as with organic acids, such as acetic acid, tartaric acid, lactic acid, citric acid and others. The acid addition compounds with organic acids, however, are more readily hydrolyzed than those with inorganic acids. Therefore, it is advisable to use a certain excess of said organic acids in aqueous solutions of such salts.

The new compounds are also capable of forming salts with inorganic and organic bases. Stable aqueous solutions can, for instance, be obtained with diethylamine, piperidine, ethylene diamine and the like. As inorganic bases there are especially suitable for salt formation the alkali hydroxides, such as sodium hydroxide and potassium hydroxide. Ammonia and alkali carbonates cannot be used since they precipitate the bases from their salt solutions.

As stated above, the new compounds are of high vasolytic activity and have a very favorable effect upon the circulatory system. Said compounds cause a decrease in the total peripheral resistance and increase in the peripheral blood flow. Following there are given some pharmacological and clinical data for a number of compounds according to the present invention.

The toxicity of 8-n-dibutylamino methyl theophylline (DI, 50) amounts to 210 mg./kg. on subcutaneous administration and 120 mg./kg. on intravenous administration to white mice. On oral administration it was tolerated in doses of 1500 mg./kg. or even higher. Such a dose is usually without any toxic effect and might sometimes merely cause slight spasms for a short period of time.

Said 8-n-dibutylamino methyl theophylline and other compounds according to the present invention are administered to human beings, preferably in the form of their acid addition products, such as the hydrochloride, the hydrobromide, the sulfate and the like, by injection or orally in the form of tablets, sugar-coated dragees or the like preparations. The dosage administered is between 100 mg. and 400 mg. of the hydrochloride of said bases, an amount which corresponds to 50-200 mg. of theophylline. To prepare an injectable preparation said amount is dissolved in 2 cc. of water or saline

solution. Tablets or dragées contain preferably 100 mg. of the hydrochloride of said bases, corresponding to about 50 mg. of theophylline.

The new salt-forming compounds according to the present invention represent a substantial advance in the art, since they permit injection of a stable solution of said drug without the addition of agents which serve as dissolving mediators. Such mediators, for instance, ethylene diamine, as is known, produce certain side reactions which are often very undesirable.

The action of 8-n-dibutylamino methyl theophylline and other compounds according to the present invention on the circulatory system can be demonstrated in animal experiments on rats by an increase of the output of the heart, i.e., the volume of circulation, and the minute volume (increase of the blood pressure amplitude), and by an increase of the functional capacity of the heart. This effect, however, does not persist as long as the decrease in peripheral resistance and in the elasticity coefficient which are also observed on administration of said drug. Furthermore, in all such experiments the diastolic blood pressure is lowered while occasionally a slight rise in systolic blood pressure occurs. When administering higher doses, the blood pressure depressing effect is prolonged.

Evaluation of said drugs on dogs after preliminary narcosis with morphine and pernocton according to the method of Wezler and Roeger, "Ergebnisse der Physiologie," vol. 41, page 292 (1939) shows that they are far superior to theophylline and its most widely used derivatives in their action upon the circulatory system when administered in equivalent dosages. Some of said compounds have a stimulating effect similar to that of theophylline upon the respiratory center. This can very readily be demonstrated after damaging the respiratory center by means of morphine. A number of said new drugs are also capable of eliminating or checking bronchospasms. A dose of 1 mg./kg. administered intravenously is sufficient to produce such spasmodic effects in animal experiments. A higher dose even increases said effect.

A very important advantage of the new drugs is their capacity of staying in solution in the gastric juice even when they remain in the stomach for several hours. In contrast hereto, theophylline is precipitated from its commercial preparations which usually contain basic agents to increase its solubility, by the gastric juice to 75% and by the intestinal juice to 50%. Thereby the pH of the gastric juice is increased from 3.0 to 8.0 and even up to 8.9. The new compounds, in contrast hereto, do not substantially affect the pH-value of the gastric juice. This explains why, for instance, 8-n-dibutylamino methyl theophylline and the like compounds can be reliably administered even on oral application and why they are much more compatible than other theophylline preparations.

The new drugs exhibit in animal experiments a remarkable diuretic effect which is especially pronounced on oral administration. Their diuretic effect surpasses even that of theophylline-sodium theophylline.

Some of said new compounds, for instance, 8-n-dibutylamino methyl theophylline, exhibit in a concentration as low as 1:20,000, marked amplification of the muscle performance when tested on the diaphragm of rats.

The stimulating effect of said drug upon the central nervous system is demonstrated in animal experiments by an increase in motility.

In general, the compounds according to the present invention exhibit low toxicity on oral administration and other favorable pharmacological properties. Most of them are non-toxic on oral administration, when given to rats, in a dose of 1.0-1.5 g./kg.

The action of the new theophylline derivatives upon the circulatory system is in most cases very impressive and in many cases at least equal to that of a solution of

theophylline in sodium theophylline. Especially effective with respect to a persistent depression of the blood pressure are 8-n-dibutylamino methyl theophylline, α-(8-dibutylamino) ethyl theophylline, and 8-benzyl cyclohexylamino methyl theophylline.

About 4-5 mg./kg. of body weight are sufficient in order to neutralize the damaging effect of morphine upon respiration whereby, for instance, 8-n-dibutylamino methyl theobromine is about as effective as 8-n-dibutylamino methyl theophylline and 8-n-dibutylamino ethyl theophylline.

The most effective and therapeutically most useful compound of this series of compounds is the 8-n-dibutylamino ethyl theophylline of Example 18. Said compound, as has been found in pharmacological as well as clinical experiments, exhibits excellent diuretic effects, highly favorable actions on the circulatory system, and a marked direct amplification of the muscle performance while it does not substantially affect the central nervous system nor increase motility nor causes undesired stimulation of respiration. The hydrochloride of said α-(8-di-n-butylamino) ethyl theophylline is the most preferred salt of said compound. It is obtained by dissolving said base in absolute ethanol and passing gaseous hydrochloric acid into said solution.

The diuretic effect of the new preparations and especially of that of the most effective 8-n-dibutylamino ethyl theophylline is at least equal and usually superior to that of the highly effective theophylline-sodium theophylline.

As stated above, the new compounds are preferably orally administered in the form of tablets, pills, powders, capsules, solutions, emulsions, suspensions, dispersions and in any other suitable form. They are preferably not used as such but are diluted with suitable diluting agents, thus, allowing better and more economical use to be made thereof.

In the case of powders, fine, uniform dispersion of the active compound within the diluting agent is of importance. Such a fine dispersion can be achieved, for instance, by mixing and milling the new compounds with a solid, pulverulent extending agent to the desired degree of fineness or by impregnating the already milled, finely powdered, solid carrier with a solution of the active compound in water or a water miscible solvent and then removing the water or solvent.

As solid carriers which are suitable for the manufacture of pulverulent preparations, various inert pulverulent distributing agents may be employed.

When preparing tablets, pills, powders and the like, the commonly used diluting agents, binders and the like are employed, such as sugar, lactose, talcum, starch, pectins, and as binders, gelatin, gum arabic, methyl cellulose, yeast extract, agar, tragacanth and others.

For preparing tablets of 8-n-dibutylamino methyl theophylline for instance, may be used the following mixture:

	G.
8-n-dibutylamino methyl theophylline.....	0.100
Starch	0.070
Talcum	0.030

Pro one tablet..... 0.200

For preparing tablets of 8-n-dibutylamino ethyl theophylline is mentioned the following mixture:

	G.
8-n-dibutylamino ethyl theophylline	0.100
Starch	0.060
Lactose	0.020
Talcum	0.020

Pro one tablet..... 0.200

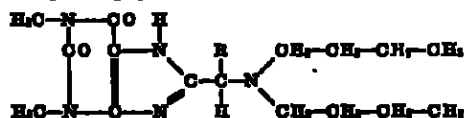
I claim:

1. 8-di-n-butylamino methyl theophylline.
2. 8-n-di-(n-butylamino) ethyl theophylline.

3. The hydrochloride of 8-di-n-butylamino methyl theophylline.

4. The hydrochloride of 8-di-n-butylamino ethyl theophylline.

5. An 8-di-n-butylamino alkyl theophylline compound selected from the group consisting of an 8-di-n-butylamino lower alkyl theophylline of the formula



wherein R is a member selected from the group consisting of hydrogen and the methyl radical, and its acid addi-

tion salts, the acid component thereof being a substantially non-toxic acid.

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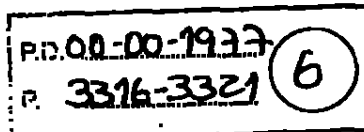
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Selective Inhibition of Cyclic Nucleotide Phosphodiesterases by Analogues of 1-Methyl-3-isobutylxanthine†

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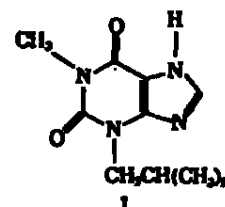
ABSTRACT: A series of 8-substituted 1-methyl-3-isobutylxanthines and 1,3-disubstituted xanthines was designed and prepared in an attempt to increase the specificity of the 1-methyl-3-isobutylxanthine (MIX) structure for one of two forms of cyclic nucleotide phosphodiesterase isolated from pig coronary arteries. The 8-substituted derivatives were in general much less potent than MIX as inhibitors of peak II phosphodiesterase activity; if the 8-substituent did not withdraw electrons, the compounds were as potent or more potent than MIX as inhibitors of peak I phosphodiesterase activity. 1-Isobutyl-3-isobutylxanthine and 1-*n*-amyl-3-isobutylxanthine, on the other hand, were about equipotent with MIX as inhibitors of peak II phosphodiesterase activity but were $\frac{1}{10}$ to $\frac{1}{5}$

as potent as MIX as inhibitors of peak I phosphodiesterase activity. Since different cell types may contain different forms of phosphodiesterase or different ratios of the forms, the xanthines were studied as inhibitors of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate hydrolysis by crude extracts from pig sperm, sea urchin sperm, and pig coronary arteries. 1-Isobutyl-3-isobutylxanthine is about twofold more potent as an inhibitor of cAMP than of cGMP hydrolysis by the coronary artery supernatant fraction, but is three- to fourfold more potent as an inhibitor of cGMP than cAMP hydrolysis by the supernatant fraction from a homogenate of pig sperm. Thus the selectivity of these inhibitors may vary from one cell type to another.

Adenosine 3',5'-monophosphate (cAMP) has been implicated as an intracellular mediator of the actions of many hormones in a variety of tissues (Robison et al., 1971) and an increasing body of evidence suggests a regulatory role for this nucleotide in vascular and other smooth muscle (Anderson et al., 1972; Triner et al., 1971). The levels of guanosine 3',5'-monophosphate (cGMP) are increased by cholinergic and other agents that produce contraction of smooth muscle (Lee et al., 1972; Schultz et al., 1973a-c; Goldberg et al., 1973) but the significance of these changing levels of cGMP is not clear. 1-Methyl-3-isobutylxanthine (MIX, 1), a potent inhibitor of cyclic nucleotide phosphodiesterases in many tissues including coronary arteries (Wells et al., 1975a), produces relaxation of smooth muscle but increases the levels of both cAMP and cGMP in these tissues (Schultz et al., 1973b; Sutherland et al., 1973).

We have separated and partially characterized cyclic nucleotide phosphodiesterase activities from the intima + media layer of pig coronary arteries (Wells et al., 1975b) and from pig and sea urchin sperm (Wells and Garbers, 1976). Two fractions of phosphodiesterase activity and a heat-stable, nondialyzable activator were separated by DEAE¹-cellulose chromatography of the 40 000g supernatant fraction of pig coronary arteries. Peak I phosphodiesterase activity catalyzes the hydrolysis of both cGMP and cAMP and exhibits classical kinetic behavior with both substrates, although it has a much lower apparent K_m for cGMP (2–4 μ M) than for cAMP (40–100 μ M). Peak I activity is stimulated three- to eightfold by a heat-stable, nondialyzable activator, while peak II activity

is unaffected by this material. Peak II exhibits apparent negatively cooperative kinetic behavior and is relatively selective for cAMP (the lower limit of the apparent K_m value, which can be only roughly estimated from curvilinear plots, is approximately 1 μ M for cAMP). The activity of peak II with cGMP as a substrate is too low to permit practical kinetic studies:



We have recently shown that MIX (1) shows some selectivity for inhibition of cGMP over cAMP hydrolysis by crude phosphodiesterase preparations from pig coronary arteries and for peak I phosphodiesterase activity over peak II (Wells et al., 1975a). In addition, we have shown that substitution on the 7-position of MIX greatly reduces the potency of the xanthine to inhibit peak II activity. One 7-substituted derivative, 7-benzyl-MIX (2), is about 25-fold more potent as an inhibitor of peak I with 1 μ M cGMP as substrate than of peak II activity with 1 μ M cAMP as substrate (Garst et al., 1976).

It has been reported that some 8-substituted theophylline analogues are more potent than theophylline as inhibitors of cyclic nucleotide phosphodiesterase activity from beef heart at high (mM) cAMP concentrations (Goodsell et al., 1971). We, therefore, studied the effect of substitution on the 8-position of MIX, which is about 30-fold more potent than theophylline as an inhibitor of cyclic nucleotide phosphodiesterase activity from pig coronary arteries. We also prepared a series of xanthines in which the substituents on the 1- and 3-positions were varied. The primary aim of the present study was to study these analogues and derivatives of MIX to ascertain if increased selectivity for inhibition of the hydrolysis of cGMP or cAMP by the separated phosphodiesterases of coronary arte-

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¹ Abbreviations used: DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; DMF, dimethylformamide.

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ries is possible. In addition we compared the potency of some of these compounds as inhibitors of the blood vessel phosphodiesterases with their potencies as inhibitors of phosphodiesterases from sea urchin sperm and pig sperm. The latter experiments were carried out because the phosphodiesterases from sperm differ from the blood vessel phosphodiesterases in several ways such as substrate specificity, distribution, and kinetic parameters (Wells and Garbers, 1976). It seemed possible that the specificity and/or potency of inhibition by the MIX derivatives would also differ in these systems.

Experimental Procedures

Melting points were determined in open glass capillary tubes using a Laboratory Devices Mel-Temp and are reported uncorrected. Infrared spectra were obtained using a Perkin-Elmer Model 257 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Jeol Model JNM-MH-100 spectrometer using tetramethylsilane as an internal standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. In all cases spectral data were consistent with the proposed structures and elemental analyses were within $\pm 0.4\%$ of the calculated values.

Enzyme Preparation. The crude supernatant and DEAE-cellulose-separated phosphodiesterase activities from pig coronary arteries were obtained as described previously (Wells et al., 1975b). Briefly, the right coronary, anterior descending, and circumflex arteries from fresh cold pig hearts were everted, and an inner layer which consisted predominantly of microscopically identifiable intima + media tissue was removed and homogenized in 4 mL of buffer (20 mM Tris-HCl, pH 7.5, 2 mM MgOAc, and 1 mM dithiothreitol) per g of tissue (wet weight). After centrifugation at 40 000g for 30 min at 0 °C, the supernatant fraction was used directly or was subjected to DEAE-cellulose chromatography as described previously (Wells et al., 1975b).

Sea urchin (*Strongylocentrotus purpuratus*) and pig sperm were collected and washed as previously described (Wells and Garbers, 1976; Garbers et al., 1971) and suspended to 20% (wet w/v) in a solution containing 20 mM Tris-HCl buffer at pH 7.5, 1 mM dithiothreitol, and 2 mM MgCl₂. This suspension was then homogenized with an Ultra-Turrax homogenizer (Jahnke and Kunkel, Staufen, Germany). The homogenate was either used directly or was centrifuged at 40 000g for 30 min at 0–4 °C.

Phosphodiesterase Assay. The compounds were assayed for inhibitor activity against the crude homogenate, the 40 000g supernatant fraction, or the separated forms of phosphodiesterase. The assay procedures have been reported (Wells et al., 1975a,b). Assays were performed with 1 μ M substrate at 30 °C for 30 min at enzyme dilutions which gave 10–20% hydrolysis of substrate in the absence of inhibitor. MIX (10^{-3} M) was included in each experiment to assure that the enzyme preparation was responding in a constant manner. The compounds were dissolved in 30% Me₂SO, and 25 μ L of this solution was added to the assay tube (final volume was 250 μ L). All activities (including control and no-enzyme blank) were measured in the presence of 3% Me₂SO, and product accumulation was linear for at least 30 min under the conditions of the assay. None of the compounds altered the efficacy of the nucleotidase step or subsequent steps in the assay. Concentrations of the compounds that inhibited by 50% the hydrolysis of 1 μ M substrate (I_{50}) were determined from concentration-percent inhibition curves, utilizing concentrations of the compounds from 10^{-7} to 10^{-6} M (or 10^{-7} to 10^{-3} M if the compound was sufficiently soluble). The presence or

absence of the calcium-binding protein activator and/or calcium ion did not affect the activity of the compounds studied. Each compound was assayed in the presence and absence of the protein activator. Since no differences were observed, the values given in Table II are composites of data obtained in the presence and absence of the activator.

Synthesis. 1,3-Disubstituted Xanthines. General Procedures. The appropriate 1-monosubstituted 6-aminouracil was prepared and alkylated following the procedure of Papeach and Schroeder (1953) using the indicated alkylating agent (Table I) to give the 1,3-disubstituted 6-aminouracil. This was converted to the xanthine using the following modification of the method of Speer and Raymond (1953). The crude 1,3-disubstituted 6-aminouracil (5–10 mmol) was suspended or dissolved with warming in 20% HOAc (25 mL). Sodium nitrite (15 mmol) in H₂O (5 mL) was added dropwise to give the 5-nitrosouracil as a red or purple precipitate. The crude 5-nitrosouracil was dissolved by warming in concentrated NH₄OH (10 mL), and Na₂S₂O₄ (30 mmol) in H₂O (20 mL) was added slowly with stirring and warming. The mixture was warmed and stirred for ca. 30 min and then cooled in an ice bath. The resulting precipitate was separated by filtration and air-dried for a short time. If no precipitate was obtained, the cold mixture was extracted with CH₂Cl₂. The CH₂Cl₂ solution was dried (MgSO₄) and then evaporated to give the 1,3-disubstituted 5,6-diaminouracil. This crude product was dissolved in 98% formic acid (10 mL) and heated under reflux for 1 h. The formic acid was removed in vacuo. To the resulting oil was added 10% NaOH (10 mL), and the mixture was heated under reflux for ca. 15 min. The reaction mixture was cooled in an ice bath and acidified with concentrated HCl. The resulting precipitate was separated by filtration and purified by recrystallization from the solvent indicated in Table I or sublimed to give compounds 3–9.

8-Substituted 1-Methyl-3-isobutylxanthines. General Procedure. 1-Isobutyl-3-methyl-5,6-diaminouracil (Papeach and Schroeder, 1952) (10 mmol) was heated under reflux in acetic anhydride (25 mL, 2 h), trifluoroacetic anhydride (25 mL, 2 h), or with pivaloyl chloride (12 mmol) in pyridine (25 mL, 6 h). The resulting solid (after washing with H₂O (25 mL) to remove pyridine salts where necessary) was dissolved with heating and stirring in 10% NaOH (50 mL). The resulting solution was filtered and allowed to cool. The solution was acidified to pH 3–4 by addition of 6 N HCl with rapid stirring. The solid was collected by filtration and washed with H₂O (25 mL) to give 10, 12, or 11, respectively. The products were then recrystallized from the solvent indicated in Table I.

8-Substituted 1-Methyl-3-isobutyl-7-benzylxanthines. General Procedure. Benzylation of 10, 11, 12, and 17 was carried out according to the previously reported method (Garst et al., 1976) by treatment with benzyl bromide and K₂CO₃ in DMF for 2 h. The reaction mixture was then poured into H₂O and cooled overnight to give 13, 14, 15, and 16, respectively. The products were recrystallized from the solvent indicated in Table I.

1-Methyl-3-isobutyl-8-bromoxanthine (17). Bromine (5 mmol) was added to a solution of 1-methyl-3-isobutylxanthine (5 mmol) and NaOAc (5 mmol) in glacial HOAc (57.5 g) heated at 40–50 °C. Heating was continued for 20 min and the solution was allowed to cool and the crude product collected by filtration. Purification was carried out by dissolution of the crude product in base followed by reprecipitation by addition of acid. The resulting solid was recrystallized from EtOH-H₂O: 95%; mp 228–229 °C. Anal. (C₁₀H₁₃BrN₄O₂) C, H.

1-Benzyl-3-isobutylxanthine (18). To a solution of 1-isobutyl-3-methylxanthine (5 mmol) and NaOAc (5 mmol) in glacial HOAc (57.5 g) heated at 40–50 °C. Heating was continued for 20 min and the solution was allowed to cool and the crude product collected by filtration. Purification was carried out by dissolution of the crude product in base followed by reprecipitation by addition of acid. The resulting solid was recrystallized from EtOH-H₂O: 95%; mp 228–229 °C. Anal. (C₁₀H₁₃BrN₄O₂) C, H.

TABLE I: Preparative Data and Physical Properties of Substituted Xanthines

No.	R ₂	R ₁ X	R ₃	Mp	Recrystn solvent or method	Yield (%)	Formula	Anal.
3	Benzyl	(None) R ₁ = H	H	314d	Acidify basic solution	41 ^a	C ₁₁ H ₁₄ N ₄ O ₂	C, H
4 ^b	Benzyl	Me ₂ SO ₄	H	261.5–263.5	EtOH–H ₂ O	32 ^a	C ₁₁ H ₁₂ N ₄ O ₂	C, H
5 ^b	Isobutyl	Me ₂ SO ₄	H	186–187.5	Subl 145 °C (1 mm)	30 ^a	C ₁₁ H ₁₄ N ₄ O ₂	C, H
6	<i>i</i> -Bu	<i>i</i> -BuOTf ^c	H	187–189	Benzene	5 ^a	C ₁₃ H ₁₈ N ₄ O ₂	C, H
7	<i>i</i> -Bu	<i>n</i> -AmOTf ^c	H	181.5–183.5	Benzene	18 ^a	C ₁₄ H ₂₀ N ₄ O ₂	C, H
8	<i>i</i> -Bu	<i>i</i> -AmOTf ^c	H	189–191	Benzene	12 ^a	C ₁₄ H ₂₀ N ₄ O ₂	C, H
9	<i>i</i> -Bu	<i>n</i> -OctOTf ^c	H	150–151	Column chromatography ^d	2 ^a	C ₁₇ H ₂₄ N ₄ O ₂	C, H
10	<i>i</i> -Bu	Me ₂ SO ₄	Me	221.5–223	EtOH–H ₂ O	65 ^e	C ₁₁ H ₁₆ N ₄ O ₂	C, H
11	<i>i</i> -Bu	Me ₂ SO ₄	<i>i</i> -Bu	208.5–210	EtOH–H ₂ O	68 ^e	C ₁₄ H ₂₂ N ₄ O ₂	C, H
12	<i>i</i> -Bu	Me ₂ SO ₄	CF ₃	216–217	EtOH–H ₂ O	52 ^e	C ₁₁ H ₁₁ F ₃ N ₄ O ₂	C, H
13	<i>i</i> -Bu	Me ₂ SO ₄	Me	149–150.5	95% EtOH	80 ^f	C ₁₀ H ₁₂ N ₄ O ₂	C, H
14	<i>i</i> -Bu	Me ₂ SO ₄	<i>i</i> -Bu	135.5–137	95% EtOH	78 ^f	C ₁₃ H ₁₈ N ₄ O ₂	C, H
15	<i>i</i> -Bu	Me ₂ SO ₄	CF ₃	87–89	95% EtOH	95 ^f	C ₁₁ H ₁₁ F ₃ N ₄ O ₂	C, H
16	<i>i</i> -Bu	Me ₂ SO ₄	Br	132.5–134	95% EtOH	82 ^f	C ₁₁ H ₁₃ BrN ₄ O ₂	C, H

^a Yield from 1-alkyl-6-aminouracil. ^b Previously prepared but physical data not given (Woodridge and Slack, 1962). ^c OTf = *p*-toluenesulfonate. ^d Neutral alumina eluted with benzene then Et₂O–MeOH. ^e Yield from 1-methyl-3-isobutyl-5,6-diaminouracil. ^f Yield for benzoylation step from appropriate xanthine.

butyl-6-aminouracil (Papesch and Schroeder, 1953) (87 mmol) in concentrated H₂SO₄ (15 mL) was added fuming HNO₃ (3.2 mL) dropwise with stirring (Brown et al., 1955). The solution was cooled on an ice bath and then poured onto ice. The precipitate, 1-isobutyl-5-nitro-6-aminouracil, was separated by filtration, air-dried, and used without further purification: 80%. A solution of this compound (10 mmol), KOH (11 mmol), and benzyl chloride (12 mmol) in DMF (15 mL) was heated at 100 °C for 1 h. The mixture was allowed to cool and the NaCl precipitate was removed by filtration. The filtrate was evaporated in vacuo to give the crude alkylated product. This crude product was reduced with Na₂S₂O₄, formylated, and cyclized according to the general procedure for 1,3-disubstituted xanthines to give 1-benzyl-3-isobutyl-xanthine: 20%. An analytical sample was obtained by sublimation (145 °C, 1 mm): mp 175–176 °C. Anal. (C₁₆H₁₈N₄O₂) C, H.

1,9-Dimethyl-3-isobutylxanthine (20): 1-Isobutyl-3-methyl-5,6-diaminouracil was prepared following the general procedure for 1,3-disubstituted xanthines through the reduction step with Na₂S₂O₄. This product was converted to 1,9-dimethyl-3-isobutyl-8-mercaptopurine by the method of Blicke and Schaaf (1956). This compound (2 mmol) was dissolved in glacial HOAc (10 mL) with warming and NaNO₂ (29 mmol) in H₂O (20 mL) was added dropwise with stirring. The reaction mixture was made basic (pH 9) with NH₄OH and diluted with water to 100 mL total volume. The solution was cooled overnight to give a crystalline precipitate. The solid was separated by filtration and washed with water: 80%; mp 164–166.5 °C. Anal. (C₁₁H₁₆N₄O₂) C, H.

Results and Discussion

The most demanding assessment of potency and selectivity of inhibition of the coronary artery phosphodiesterases is to compare the potency of a compound as an inhibitor of cGMP hydrolysis by peak I with the potency of the compound as an inhibitor of cAMP hydrolysis by peak II, since these are the high-affinity substrates for the respective enzyme preparations (Wells et al., 1975b). The 1 μM substrate concentration used in this study is near the apparent *K_m*'s of the enzymes being studied. All of the compounds in this study were competitive inhibitors of peak I phosphodiesterase activity. Although *K_i* values could be obtained, the potencies are given as *I*₅₀ values (the concentrations required to inhibit by 50% the hydrolysis of 1 μM substrates), because the purpose of the study was to compare the potencies of the compounds as inhibitors of peak I activity with the potencies to inhibit peak II. Since peak II displays anomalous kinetics, it is difficult or impossible to estimate meaningful *K_i* values from graphical analysis of the data (Wells et al., 1975a).

A number of generalizations are suggested by the data in Table II concerning structural requirements for potency of xanthines as inhibitors of pig coronary artery phosphodiesterases. It would appear that alkyl substitution larger than methyl in position 3 of the xanthine nucleus enhances potency against either peak of activity. Some substitution other than H at position 1 is apparently also required for potent inhibition of either form of phosphodiesterase. Substitution of electron-withdrawing groups at position 8 reduces the potency against either peak. Alkyl substitution at position 7 (Garst et al., 1976) or 8 has little effect on potency against peak I but greatly re-

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TABLE II: Inhibition of Separated Phosphodiesterases from Pig Coronary Arteries.

R1N1C(=O)N(C(=O)N2C(=O)N(R4)C(R3)=N2)C(=O)N1R2

Compound No.	R ₁	R ₂	R ₃	R ₄	I ₅₀ (μM) ^a and % inhibition at 100 μM		
					Peak I		Peak II cAMP
					cGMP	cAMP	
1 ^b	CH ₃	<i>t</i> -Bu	H	H	6.3 ± 0.7 (6)	3.9 ± 0.3 (6)	15 ± 2 (3)
10	CH ₃	<i>t</i> -Bu	H	CH ₃	1.9 ± 0.1 (5)	2.3 ± 0.6 (7)	68 ± 4 (6)
12	CH ₃	<i>t</i> -Bu	H	CF ₃	>100 (38% ± 5) (6)	84 ± 5 (6)	>100 (21% ± 5) (3)
11	CH ₃	<i>t</i> -Bu	H	C(CH ₃) ₃	1.8 ± 0.2 (7)	1.2 ± 0.2 (9)	58 ± 6 (6)
17	CH ₃	<i>t</i> -Bu	H	Br	55 ± 9 (8)	35 ± 4 (6)	>100 (24% ± 5) (4)
2 ^b	CH ₃	<i>t</i> -Bu	Benzyl	H	4.4 ± 0.7 (7)	3.6 ± 0.8 (7)	108 ± 14 (4)
13	CH ₃	<i>t</i> -Bu	Benzyl	CH ₃	2.2 ± 0.3 (10)	3.2 ± 0.6 (10)	24 ± 4 (4)
14	CH ₃	<i>t</i> -Bu	Benzyl	C(CH ₃) ₃	6.2 ± 0.6 (7)	6.0 ± 0.4 (7)	>10
15	CH ₃	<i>t</i> -Bu	Benzyl	CF ₃	11 ± 1 (6)	17 ± 4 (6)	78 ± 6 (5)
16	CH ₃	<i>t</i> -Bu	Benzyl	Br	2.2 ± 0.4 (6)	2.9 ± 0.5 (6)	16 ± 1 (3)
19 ^c	H	<i>t</i> -Bu	H	H	>100 (43% ± 6) (5)	>100 (34% ± 2) (6)	>100 (58% ± 3) (4)
6	<i>t</i> -Bu	<i>t</i> -Bu	H	H	>100 (41% ± 7) (5)	>100 (38% ± 4) (5)	>100 44 ± 7 (3)
7	<i>n</i> -Amyl	<i>t</i> -Bu	H	H	>100 (46% ± 5) (10)	>100 (42% ± 4) (12)	>100 40 ± 10 (4)
8	isoamyl	<i>t</i> -Bu	H	H	86 ± 5 (7)	>100 (44% ± 2) (7)	>100 17 ± 3 (6)
9	<i>n</i> -Octyl	<i>t</i> -Bu	H	H	>100 (33% ± 6) (6)	>100 (29% ± 7) (6)	>100 (38% ± 2) (3)
18	Benzyl	<i>t</i> -Bu	H	H	22 ± 4 (3)	25 ± 8 (3)	36 ± 8 (6)
21	isoamyl	CH ₃	H	H	>100 (43% ± 3) (4)	>100 (54% ± 4) (6)	>100 50 ± 9 (4)
Theophylline ^d	CH ₃	CH ₃	H	H	100 ± 9	58 ± 4	190 ± 6
4	CH ₃	Benzyl	H	H	26 ± 5 (7)	20 ± 3 (7)	32 ± 4 (4)
5	CH ₃	isoamyl	H	H	6.0 ± 1 (4)	6.3 ± 2 (5)	17 ± 2 (4)
3	H	Benzyl	H	H	>100 (26% ± 3) (8)	>100 (30% ± 2) (8)	>100 90 ± 8 (4)
20	CH ₃	<i>t</i> -Bu	9-CH ₃	H	>100 (38% ± 4) (6)	>100 (55% ± 5) (5)	>100 (9.0% ± 2) (3)

^a I₅₀ value is the concentration (μM) of agent required to inhibit by 50% the hydrolysis of 1 μM substrate. Values are ± standard errors of the number of determinations given in parentheses. Some of the agents were insoluble above 100 μM; therefore, % inhibition at 100 μM is given if I₅₀ is above 100 μM. ^b Garri et al. (1976). ^c Wooldridge and Slack (1962). ^d Wells et al. (1973a).

duces potency against peak II. Substitution of certain saturated alkyl groups larger than methyl at position 1 has little effect on potency against peak II but greatly reduces potency against peak I. These generalizations would indicate that there are relatively strict electronic requirements at the site of inhibition of both forms of phosphodiesterase and that both forms have rather specific requirements for substitution at position 3 of the xanthine nucleus. Further, there appears to be a tolerance or preference for alkyl bulk in positions 7 or 8 at the peak I site that is absent at the peak II site. Alkyl bulk in position 1, however, is tolerated at the peak II site but not by peak I.

Substitution of a benzyl (4), isoamyl (5), or isobutyl (1) moiety for the methyl group in the 3-position of theophylline brings about a 4- to 16-fold increase in potency against either peak of activity. Comparison of MIX (1) or 4 with 19 and 3, respectively, shows that replacement of the 1-methyl group with H reduces potency against either peak from 3- to 16-fold. Such a replacement would be expected to bring about relatively large changes in the electronic and tautomeric properties of the xanthine ring. Another indication of the importance of the electronic properties of the xanthine is the effect of substitution

of electron-withdrawing groups in the 8-position. The methyl and trifluoromethyl groups are quite similar in size (van der Waals radii of H and F are 1.2 and 1.35 Å, respectively; Hine, 1956) but they have distinctly different electronic properties (the pK_a of acetic and trifluoroacetic acid are 4.76 and 0.23, respectively; Albert and Serjeant, 1962). Thus, the 8-trifluoromethyl group of 12 has a much greater tendency to withdraw electrons from the xanthine nucleus than does the 8-methyl group of 10. A comparison of the I₅₀ values in Table II for 8-methyl-MIX (10) and 8-trifluoromethyl-MIX (12) shows the greater potency of 10 against either peak of activity. Similar results are found with an 8-bromo group (17) which is also electron withdrawing and is slightly smaller in size than methyl (van der Waals radii for bromo and methyl are 1.95 and 2.0 Å, respectively; Hine, 1956). This effect of electron-withdrawing groups is, for reasons not immediately obvious, attenuated or eliminated if a benzyl moiety is substituted in the 7-position (Table II, compounds 15 and 16).

8-Methyl-MIX (10) and 8-*tert*-butyl-MIX (11) are about one-fourth as potent as MIX as inhibitors of peak II activity but are slightly more potent than MIX against peak I. The

TABLE III: Inhibition of the Cyclic Nucleotide Phosphodiesterase Activities in Crude Supernatant Fractions or Homogenate of Pig Coronary Arteries, Pig Sperm, and Sea Urchin Sperm.

No.	Compound	I_{50}^a					
		Coronary artery supernatant fraction		Pig sperm supernatant fraction		Urchin sperm homogenate	
		cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
1	MIX	11 ± 2	5.6 ± 0.8	65 ± 7	4.7 ± 0.5	5.4 ± 0.2	0.9 ± 0.2
2	7-Benzyl MIX	30 ± 6	3.9 ± 0.6	84 ± 10	4.5 ± 0.6	21 ± 2	>100
11	8- <i>t</i> -Bu MIX	18 ± 4	1.9 ± 0.4	>100	2.0 ± 0.7	7.7 ± 1.7	0.2 ± 0.01
10	8-Methyl MIX	21 ± 5	2.0 ± 1	9.8 ± 0.2	1.6 ± 0.4	1.4 ± 0.3	0.08 ± 0.01
8	1-Isomyl-3-isobutylxanthine	40 ± 4	97 ± 3	>100	28 ± 6	19 ± 1	42 ± 2

^a Values are means ± standard errors of four determinations with four different preparations.

reduction in potency as inhibitors of peak II would seem to be because the binding site for the xanthines on peak II cannot tolerate a group larger than H on the 8-position of MIX. The catalytic site of peak I, however, tolerates or prefers relatively large substituents in this position. Similarly, substitution of relatively large groups (e.g., *n*-hexyl) on the 8-position of theophylline increased the potency of the compounds as inhibitors of a beef heart phosphodiesterase as measured at high (mM) substrate concentration (Goodsell et al., 1971).

Substitution of an isobutyl, *n*-amyl, or isoamyl group for the 1-methyl group of MIX (compounds 6, 7, and 8) has a relatively small effect on potency against peak II but significantly reduces potency against peak I. Thus, while 8-methyl-MIX (10) and 8-*tert*-butyl-MIX (11) were 30- to 35-fold more potent as inhibitors of 1 μ M cGMP hydrolysis by peak I than of hydrolysis of 1 μ M cAMP by peak II, compounds 6, 7, and 8 are 2- to 5-fold more potent as inhibitors of peak II than of peak I under the same conditions. It appears that large alkyl groups in the 1-position of the xanthine molecule cannot be tolerated by the catalytic site of peak I, while the site to which the xanthines bind on peak II can accommodate a group at least as large as isoamyl. That steric size of the groups may not be the only factor that determines the effect of substitution at the 1-position is indicated by the data for 1-octyl-3-isobutylxanthine (9) and 1-benzyl-3-isobutylxanthine (18). Compound 9 is quite inactive against both peaks, indicating the limits of the tolerance for bulk in this position by peak II. The relatively large benzyl group in 9, however, has less effect on peak I inhibition than might be expected and leaves this compound equipotent with the isobutyl (6) and *n*-amyl (7) compounds against peak II. Data for 1-isoamyl-3-methylxanthine (21) suggest some affinity for the 1-isoamyl group since this compound is almost fourfold more potent against peak II than is theophylline.

We have previously shown that substitution of a benzyl group for the 7-H of MIX yields a compound (2) with increased selectivity for peak I phosphodiesterase (Garst et al., 1976). We, therefore, prepared a series of 7-benzyl 8-substituted MIX derivatives (13-16). 7-Benzyl-8-methyl-MIX (13) and 7-benzyl-8-*tert*-butyl-MIX (14) were less potent as inhibitors of peak I activity and less selective between peak I and peak II activity than were the corresponding 8-substituted compounds (10 and 11). On the other hand, substitution of a benzyl moiety for the 7-H of 8-trifluoromethyl-MIX and 8-bromo-MIX gave compounds (15 and 16) with greater potency as inhibitors of peak I activity than the corresponding 8-substituted compound (12 and 17).

9-Methyl-MIX (20), although not as potent as 7-methyl-MIX (Garst et al., 1976) as an inhibitor of peak I, has very low

potency as an inhibitor of peak II; therefore, proper substitution in the 9-position might yield compounds with high specificity for inhibition of peak I.

The use of separated forms of phosphodiesterase allows a more valid and straightforward interpretation of the selectivity and nature of inhibition than does the use of whole homogenates. However, in examining only the separated enzymes, it might be possible to overlook inhibition of significant activities that are not present among the separated forms. Moreover, the patterns of inhibition seen in crude systems may be more representative of the composite patterns that occur in intact cells. Table III gives the inhibitory potency of some of these xanthines as inhibitors of total cGMP and cAMP phosphodiesterase activities of crude 40 000g supernatant fractions from coronary arteries and pig sperm and in the whole homogenate of sea urchin sperm. Whole homogenate of sea urchin sperm was used because the phosphodiesterase activity is predominantly located in the particulate fraction, whereas most of the activity of the coronary arteries and pig sperm is in the supernatant fraction (Wells et al., 1975b; Wells and Garbers, 1976). At 1 μ M cAMP, total phosphodiesterase activity of peak I from coronary artery is approximately equal to the total activity of peak II; therefore, a compound which selectively inhibits peak I will have a significant effect on the hydrolysis of cAMP by the crude supernatant fraction. This point is demonstrated by a comparison of the data in Table II with the data of Table III.

The relative potencies of these compounds to inhibit cAMP and cGMP hydrolysis changes or even reverses from the coronary artery system to pig or sea urchin sperm systems. For example, 7-benzyl-MIX (2), which is about eightfold more potent as an inhibitor of cGMP than cAMP hydrolysis by the crude artery supernatant fraction, is at least fourfold more potent as an inhibitor of cAMP than cGMP hydrolysis by the crude sea urchin sperm homogenate (Table III).

The data in Table III indicate that these compounds are, in general, much more potent as inhibitors of sea urchin sperm phosphodiesterase activities than activities from the two other sources studied and that 8-*tert*-butyl-MIX (11) and 8-methyl-MIX (10) are extremely potent (I_{50} = 0.2 and 0.08 μ M, respectively) inhibitors of 1 μ M cGMP hydrolysis by the crude preparation from sea urchin sperm.

The data presented demonstrate that modification of the xanthine molecule can give rise to highly selective inhibitors of cyclic nucleotide phosphodiesterases. The data also emphasize that the selectivity and/or potency of a given compound for inhibition of the hydrolysis of cAMP or cGMP may vary from one cell type to another. Therefore, any studies that rely upon the pharmacological manipulation of cyclic nucle-

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otide levels by phosphodiesterase inhibition should be based upon a knowledge of the potency of the agent to inhibit the phosphodiesterase activities of the cell type being studied.

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SUPERINTENDENCIA

**REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT**

PATENTE DE INVENCION ☒

MODELO DE UTILIDAD []

- ◆ Indicación de que se solicita la concesión de una patente [☒]
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud [☒]
- ◆ Descripción de la invención [☒]
- ◆ Dibujos de ser estos pertinentes [☒]
- ◆ Comprobante de pago de las tasas establecidas [☒]

COMPLETA ☒

INCOMPLETA []

DISEÑO INDUSTRIAL []

- ◆ Indicación de que se solicita el registro de Diseño Industrial []
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ◆ Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño []
- ◆ Comprobante de pago de las tasas establecidas []

COMPLETA []

INCOMPLETA []

ESQUEMA DE TRAZADO []

- ◆ Indicación de que se solicita el registro de un Esquema de Trazado []
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ◆ Representación gráfica del esquema trazado []
- ◆ Comprobante de pago de las tasas establecidas []

COMPLETA []

INCOMPLETA []

Fdo B
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REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
RAISBECK J. IAN
Apoderado(a) y/o Representante de
SMITHKLINE BEECHAM CORPORATION

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	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	6572

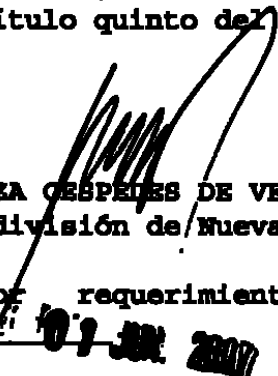
Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.

2. El solicitante deberá aclarar el numero de reivindicaciones , por cuanto el número de presentadas en el idioma original, no coinciden con la traducción presentada en fase nacional.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.


ALIX CARMENZA GONZALEZ DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de
fecha 01 JUN 2007
adpa11