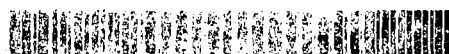


Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

E. _____ S. _____

SECRETARIA DE INDUSTRIA Y COMERCIO



Nº 04-085271-00007-0000

2020 NUECREACIONES
2019 FASENACIONAL II
Folios: 91

Re: **P1.-MERCK FROSST CANADA LTD. Y AXYS PHARMACEUTICALS, INC.-Solicitud de Patente de Invención en Fase Nacional PCT para "INHIBIDORES DE LA CISTEÍN-PROTEASA CATEPSINA".-Clase A61K.-Expediente número 04-085.271.-**

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 2 de Noviembre de 2007 y a mi memorial de plazo de Enero 28 de 2008.

2. Con el fin de dar respuesta a las objeciones presentadas por el Señor Examinador en su Concepto Técnico No. **1975** sobre la patentabilidad de la presente solicitud en lo referente a claridad, unidad de invención, excepciones a la patentabilidad y falta de novedad, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos, por medio de los cuales se superan completamente dichas objeciones.

3. **Claridad De la Solicitud.**

3.1 **Descripción.**

El señor Examinador objeta la claridad de la descripción de la presente invención señalando que *"la invención no se encuentra sustentada en el aparte descriptivo de la solicitud"*. El Señor Examinador resalta que no se encuentran datos para los ensayos de inhibición de Catepsinas K, S, B y L, y que *"no se observa ninguna ventaja sobre los compuestos del estado de la técnica"*.

Sobre ese particular me permito manifestar que tal como fue presentada, la descripción es clara al revelar los compuestos materia de la presente solicitud, como me permito exponer a continuación:

La descripción de la solicitud en estudio claramente enseña nuevos compuestos que tienen actividad inhibitoria frente a catepsinas. Mas aun, como se ha presentado dicha descripción la persona versada en la materia podría entender qué tipos de compuestos son considerados en la invención, cómo obtenerlos y su utilidad como inhibidores de cisteín-proteasas.

Dado el gran número de compuestos considerados (Ver por ejemplo páginas 32 a 63 de la descripción, o la reivindicación 9) es muy difícil consignar en la descripción los resultados de los ensayos de inhibición obtenidos con todos los compuestos. Sin embargo, la descripción claramente manifiesta que los compuestos aquí revelados actúan como inhibidores de catepsinas (página 63), y **es claro que dicha actividad ha sido comprobada por el solicitante**. En este punto me permito resaltar lo preceptuado en el párrafo primero del Artículo 28 de la Decisión Andina 486 de 2000, en relación con su literal c):

*"Artículo 28.- La descripción deberá divulgar la invención de manera **suficientemente clara y completa para su comprensión y para que una persona capacitada en la materia técnica correspondiente pueda ejecutarla**. La descripción de la invención indicará el nombre de la invención e incluirá la siguiente información:*

...

*c) una descripción de la invención en términos que permitan la comprensión del problema técnico y de la solución aportada por la invención, exponiendo las diferencias y **eventuales ventajas** con respecto a la tecnología anterior;"* (Resaltado fuera de texto).

A la luz del citado artículo, la descripción de la presente solicitud es **clara** toda vez que la persona versada en la materia, a partir de dicha descripción, podría **comprender** que la invención se relaciona con nuevos compuestos que sirven como inhibidores de catepsinas, y podría a partir de las especificaciones allí consignadas **realizar** dichos compuestos y comprobar su actividad inhibitoria.

En este mismo sentido, me permito también señalar lo que con respecto a la suficiencia de la descripción enseña el Manuel Andino para el Examen de Patentes:

"El propósito de la descripción es asegurar que por un lado, la solicitud contenga la suficiente información técnica para que una persona con conocimiento medio en el arte pueda poner en práctica la invención, y por el otro que esta divulgación sea suficiente para conocer el aporte que se está haciendo a la tecnología".¹

Teniendo esto en consideración, la descripción contiene elementos suficientes para que la persona con conocimiento medio en el arte pueda llegar a los compuestos revelados y claramente se muestra que el **aporte** hecho por la invención es el de **proporcionar nuevos compuestos** que presentan actividad como inhibidores de cisteín-proteasas.

De manera adicional a lo anteriormente expuesto, y para sustentar la declarada actividad en la inhibición de catepsinas de los compuestos considerados en la solicitud en estudio, muy comedidamente me permito llamar la atención de ese Despacho a los siguientes documentos, de publicación posterior a la solicitud en estudio, que recogen gran número de datos experimentales a ese respecto.

- **Documento Bioorganic & Medicinal Chemistry Letters 15 (2005) 4741-4744**. Dicho documento muestra cómo el reemplazo del enlace amídico por el grupo CF₃ en compuestos que presentan alguna actividad como inhibidores de catepsina, resulta en ventajas de potencia y selectividad en la actividad inhibitoria.

El citado documento muestra que el reemplazo del grupo carbonilo de la amida por un CF₃ hace que la no- basicidad del nitrógeno amídico se mantenga permitiendo el enlace de puente de hidrógeno del compuesto con la Glicina66 de la catepsina K, el cual es importante para la inhibición.

La tabla 1 de dicho documento muestra cómo los compuestos que incluyen un grupo CF₃, como los revelados en la presente solicitud, tienen

¹ Secretaría General de la Comunidad Andina "Manual para el examen de solicitudes de patentes de invención en las oficinas de propiedad industrial de los países de la Comunidad Andina". Lima, 2004. Página 33.

una actividad y/o selectividad mayor como inhibidores de catepsinas, comparados con sus análogos estructurales que contienen el enlace amida (siendo de 10 a 20 veces más potentes).

Me permito señalar específicamente el compuesto 8 de dicha Tabla 1, el cual es uno de los posibles estereoisómeros del ejemplo 7 de la solicitud en estudio, que muestra una selectividad alta con respecto a la inhibición de la catepsina K (hasta 10.000 veces mayor que para otras catepsinas) y un IC₅₀ muy bajo, ≤ 5 pM. Esto demuestra la efectividad de los compuestos revelados en la presente solicitud como inhibidores de catepsina y sus ventajas con respecto a los revelados en el estado de la técnica.

Table 1. Potency of inhibitors in purified enzyme assays

Compound	IC ₅₀ (nM) ^a			
	Cat K ^b	Cat B ^c	Cat L ^c	Cat S ^c
2	11	3950	3725	2010
3	1.0	123	352	102
6	1.3	3353	34	121
7	7.3	2477	84	145
8	≤0.005 ^d	1111	47	451
9	4.6	>10,000	68	902
10	0.015 ^d	344	26	120
12	0.6	1050	3903	>10,000
13	5.0	>10,000	>10,000	>10,000
14	0.4	614	2274	>10,000

^a IC₅₀s are an average of at least two independent titrations. See Ref 15 for assay conditions.

^b Humanized rabbit enzyme.

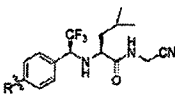
^c Human enzyme.

^d Low enzyme conditions—see Ref 12.

- Por otra parte, la actividad que presentan los compuestos considerados en la presente solicitud como inhibidores de la actividad de catepsinas es también consignada en el documento **Bioorganic & Medicinal Chemistry Letters 16 (2006) 1985–1989**. La tabla 3 del citado documento resume las actividades in Vitro de una serie de compuestos de la invención.

Dentro de esta tabla se muestra como el compuesto 1 (ejemplo 3 de la solicitud en estudio) presenta una alta selectividad frente a la catepsina K, teniendo una alta actividad inhibitoria con un IC₅₀ ≤ 50 pM. De igual forma compuestos como el ejemplo 9 de la presente solicitud (compuesto 23 en la tabla 3) o el ejemplo 6 (compuesto 27 en la tabla 3) presentan una alta actividad y selectividad en la inhibición de la catepsina K.

Table 3. In vitro activity of representative P3 analogs



Compound	R	IC ₅₀ ^a (nM)				
		Cat K ^b	Cat B	Cat L	Cat S	Bone res ^c
1	4-Piperazin-1-yl phenyl	≤0.005 ^d	1111	47	451	12 ^e
12	Phenyl	0.2	1605	1473	303	44
13	2-Fluorophenyl	0.1	6086	1670	614	28 ^e
14	3-Fluorophenyl	0.5	2476	2792	278	67 ^e
15	4-Fluorophenyl	0.6	5939	887	449	29 ^e
16	3,4-Difluorophenyl	1.1	8869	1001	189	46 ^e
17	3,5-Difluorophenyl	6	>10,000	3002	249	n.d.
18	4-Methylphenyl	0.3	1337	809	282	27 ^e
19	4-Methoxyphenyl	0.1	1660	560	196	n.d.
20	4-Cyanophenyl	0.9	7392	344	172	30 ^e
21	4-(Methylthio)phenyl	0.2	1400	524	153	50 ^e
22 ^f	4-(Methylsulfonyl)phenyl	0.2	5239	264	178	13 ^e
23	4-(Aminosulfonyl)phenyl	0.2	1561	470	154	52
24	3-(Methylsulfonyl)phenyl	0.3	6984	1480	265	53 ^e
25	Phenoxy	2.3	>10,000	771	220	88 ^e
26	Benzoyl	1.5	>10,000	1915	254	151 ^e
27	Pyridin-4-yl	0.3	2374	80	62	19
28	3-Thienyl	0.2	1155	1475	365	33 ^e
29	1,3-Thiazol-2-yl	0.3	4818	878	240	52
30	3-Methyl-1,2,4-oxadiazol-5-yl	2.6	4073	314	436	118 ^e
31	Pyrimidin-2-yl	44	5903	1857	1698	93 ^e
32	4-(1 <i>H</i> -Indol-4-yl)	0.4	4699	3047	607	80 ^e
33	4'-(Methylsulfonyl)biphenyl-4-yl	1.2	1873	2422	653	5 ^e
34	4-Pyridin-4-yl-phenyl	<0.2	483	342	188	14 ^e

^a IC₅₀s are an average of at least two independent titrations unless specified otherwise.

^b Humanized rabbit enzyme (Ref. 3), see Ref. 6 for assay conditions.

^c Rabbit bone resorption assay, see Ref. 8 for assay conditions.

^d IC₅₀ was determined at a concentration of 10 pM enzyme.

^e This value likely represents the lower limit of the assay.

^f L-873724.

^g n = 1. n.d. = not determined.

Adicionalmente, el citado documento muestra como el compuesto 22 (L-873724), que se encuentra dentro de los considerados en la presente solicitud, tiene una buena farmacocinética, presentando biodisponibilidad oral, no-lisosomatropismo y eficacia en la resorción de hueso en el modelo de monos rhesus.

- En este orden de ideas, el documento **ChemBioChem, 2006,7, 1525-1535**, consigna igualmente la actividad en la inhibición de catepsinas de los compuestos considerados en la presente invención. En la tabla 7 de dicho documento se registran datos de la actividad inhibitoria de varios compuestos considerados en la presente solicitud, frente a catepsinas K, B, L, y S, su influencia en la resorción de hueso y su comportamiento farmacocinético en ratas. En la citada tabla 7, se observa como los compuestos presentan alta actividad con respecto a las catepsinas K, L y S, mostrando una mayor selectividad con respecto a la catepsina K, con valores de IC₅₀ desde 0,9 hasta 0,2 nM. Es igualmente evidente que la presencia de una sulfona o una sulfonamida en la posición 4 del bifenilo de la molécula (compuestos 26 y 27 de la tabla 7) produce un

comportamiento farmacocinético que los hace ventajosos frente a otros compuestos conocidos en el estado del arte.

El documento además señala que, aunque las catepsinas se localizan en compartimientos ácidos, se ha demostrado que los inhibidores lisosomatrópicos no presentan una alta potencia como inhibidores de la catepsina K. Los compuestos de la presente invención, al ser inhibidores no básicos tienen una potencia alta (sub nanomolar) al inhibir dicha catepsina K, mostrando de igual forma una alta selectividad sobre otros tipos de catepsinas.

- De otro lado, un documento más reciente, **Bioorganic & Medicinal Chemistry Letters 18 (2008) 923-928**, muestra también ensayos de inhibición de catepsinas con compuestos seleccionados de la solicitud en estudio. La tabla 1 de dicho documento incluye compuestos como el ejemplo 38 de la presente solicitud (compuesto 19 en la tabla 1), el ejemplo 14 (compuesto 23 en la tabla 1) y el ejemplo 15, considerado en la reivindicación 10 de la solicitud en estudio, (compuesto 24 de la tabla 1, odanacatib). Dicha tabla confirma nuevamente la alta actividad y selectividad que presentan los compuestos de la presente invención como inhibidores de la catepsina K, con valores de IC₅₀ del orden de 0,2 nM.

El documento igualmente revela, como el compuesto 24 (odanacatib, ejemplo 15 de la solicitud en estudio) tiene una mayor selectividad a la catepsina K aún en estudios en célula completa, comparado con otros inhibidores conocidos en el estado del arte como son el balicatib y el relacatib (Tablas 2 y 3). El odanacatib presenta, además, una alta actividad en resorción de hueso, una excelente estabilidad metabólica y, debido a su selectividad, una disminución en la indeseada acumulación de colágeno en fibroblastos dérmicos (Figura 2 del citado documento); siendo así un candidato muy atractivo para el tratamiento de osteoporosis.

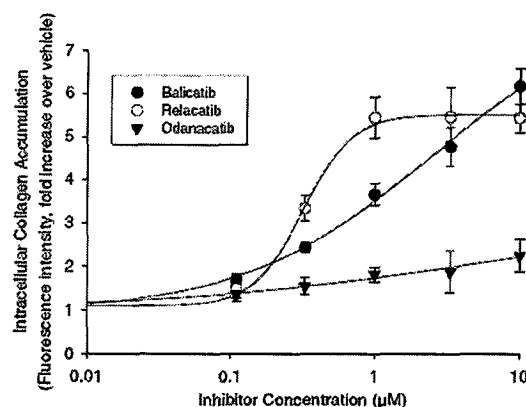


Figure 2. Effect of balicatib, relacatib, and odanacatib on the intracellular accumulation of Type I collagen in cultured primary human dermal fibroblasts. The data represent the average $\pm$ SD for at least $n = 2$ experiments for each inhibitor. A similar profile was observed using cells from a second donor.

- Dicha utilidad en el tratamiento de la osteoporosis del odanacatib, es de igual forma señalada en el documento **Clinical pharmacology & Therapeutics, Vol. 83, enero 2008,172-176**, donde se resalta que en los estudios clínicos en pacientes post-menopáusicas, el Odanacatib ha mostrado ser seguro y bien tolerado, sin presentar tendencia a efectos secundarios dependientes de la dosis, permitiendo un aumento en la densidad mineral ósea en el cuello femoral del 2,5% cuando se emplea una dosis semanal de 50mg.

Por último, me permito señalar que **todos los datos aportados anteriormente, contenidos en los documentos citados, comprueban la utilidad de los compuestos revelados en la solicitud en estudio como inhibidores de catepsinas**, soportando lo que a ese respecto enseña la descripción de la presente solicitud.

Para concluir mi argumentación en torno a la claridad de la descripción, me permito manifestar que los argumentos expuestos anteriormente han demostrado que la ésta **es clara y suficiente** al revelar la invención, toda vez que la persona versada en la materia podría, a partir de dicha descripción, comprender que la materia reclamada se refiere a nuevos compuestos que presentan actividad como inhibidores de cisteín-proteasas, y que dicha persona versada en la materia podría desarrollar dichos compuestos y reconocer su aporte al estado de la técnica a partir de las enseñanzas consignadas en dicha descripción. Sumado a lo

anterior, los documentos citados proporcionan datos que soportan las revelaciones que hace la descripción de la presente solicitud con respecto a la actividad y ventaja sobre el estado de la técnica de los compuestos aquí revelados. De esta forma, la descripción de la presente solicitud **cumple lo preceptuado en el Artículo 28 de la Decisión Andina 486 de 2000.**

3.2. **Reivindicaciones.**

De igual forma, el señor Examinador objeta la claridad del capítulo reivindicatorio señalando que existen términos muy amplios que no permiten definir los compuestos de la presente solicitud. Además presenta objeciones específicas sobre ciertas reivindicaciones por considerarlas redundantes.

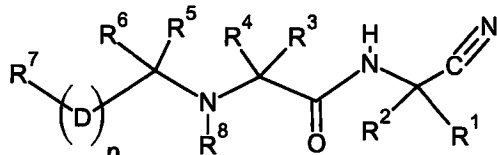
Teniendo en consideración dichas objeciones es deseo del solicitante **modificar el capítulo reivindicatorio para ajustarlo a los lineamientos de claridad preceptuados en el Artículo 30 de la Decisión 486 de 2000. De esta forma el capítulo reivindicatorio queda constituido de quince (15) reivindicaciones.**

Me permito manifestar que las modificaciones mencionadas no amplían de manera alguna el alcance de la invención toda vez que las mismas encuentran pleno sustento en la descripción de la solicitud, tal como me permito señalar a continuación:

- **Reivindicación 1.** Acatando las objeciones del Señor examinador se ha restringido el significado de R^7 al caso en el cual corresponde a hidrógeno, alquilo C_{1-6} , $-SO_2R^{12}$, $-SO(R^{12})$, $-OSO_2R^{10}$, $-SOMN(R^c)(R^d)$, $-SOMCH(R^{10})(R^{11})$, o $-SOM(R^c)(R^d)$; de igual forma y con el fin de hacer mayor claridad sobre los compuestos reclamados, se introdujo en esta reivindicación la definición de los términos "arilo" y "heteroarilo" consignadas en la descripción. Adicionalmente, por un error involuntario en la traducción de la solicitud, la correspondencia de n fue escrita como "entre un número entero de cero a tres", y no "entre un número entero de **uno** a tres" como se encuentra consignado en la reivindicación 1 de la solicitud internacional (PCT/US03/06147, página 230). Este error es corregido para ajustar la equivalencia de n a la solicitud internacional.

De esta forma, la nueva reivindicación 1 se lee como sigue:

"La presente invención se refiere a compuestos de la siguiente formula química:



en la que R^1 es hidrógeno, alquilo C_{1-6} o alquenilo C_{2-6} donde dichos grupos alquilo y alquenilo están opcionalmente sustituidos con de uno a seis halo, cicloalquilo C_{3-6} , $-SR^9$, $-SR^{12}$, $-SOR^9$, $-SOR^{12}$, $-SO_2R^9$, $-SO_2R^{12}$, $-SO_2CH(R^{12})(R^{11})$, $-OR^{12}$, $-OR^9$, $-N(R^{12})_2$, arilo, heteroarilo o heterociclilo donde dichos grupos arilo, heteroarilo y heterociclilo están opcionalmente sustituidos con uno o dos sustituyentes seleccionados independientemente entre alquilo C_{1-6} , halo, hidroxialquilo, hidroxilo, alcoxi o ceto;

R^2 es hidrógeno, alquilo C_{1-6} o alquenilo C_{2-6} donde dichos grupos alquilo y alquenilo están opcionalmente sustituidos con de uno a seis halo, cicloalquilo C_{3-6} , $-SR^9$, $-SR^{12}$, $-SOR^9$, $-SOR^{12}$, $-SO_2R^9$, $-SO_2R^{12}$, $-SO_2CH(R^{12})(R^{11})$, $-OR^{12}$, $-OR^9$, $-N(R^{12})_2$, arilo, heteroarilo o heterociclilo donde dichos grupos arilo, heteroarilo y heterociclilo están opcionalmente sustituidos con uno o dos sustituyentes seleccionados independientemente entre alquilo C_{1-6} , halo, hidroxialquilo, hidroxilo, alcoxi o ceto;

o R^1 y R^2 pueden tomarse junto con el átomo de carbono al que están unidos para formar un anillo cicloalquilo C_{3-8} o heterociclilo donde dicho sistema de anillos está opcionalmente sustituido con uno o dos sustituyentes seleccionados independientemente entre alquilo C_{1-6} , hidroxialquilo, haloalquilo, o halo;

R^3 es hidrógeno, alquilo C_{1-6} o alquenilo C_{2-6} donde dichos grupos alquilo y alquenilo están opcionalmente sustituidos con cicloalquilo C_{3-6} o de uno a seis halo;

R^4 es hidrógeno, alquilo C_{1-6} o alquenilo C_{2-6} donde dichos grupos alquilo y alquenilo están opcionalmente sustituidos con cicloalquilo C_{3-6} o de uno a seis halo;

o R^3 y R^4 pueden tomarse junto con el átomo de carbono al que están unidos para formar un anillo cicloalquilo C_{3-8} , anillo C_{5-8} cicloalqueno, o un heterociclilo de cinco a siete miembros donde dichos grupos cicloalquilo, cicloalqueno y heterociclilo están opcionalmente sustituidos con uno o dos sustituyentes seleccionados independientemente entre alquilo C_{1-6} , halo, hidroxialquilo, hidroxilo, alcoxi o ceto;

R^5 se selecciona entre hidrógeno o alquilo C_{1-6} sustituido con halo 1-6;

R^6 es arilo, heteroarilo, haloalquilo C_{1-6} , arilalquilo o heteroarilalquilo, donde dichos grupos arilo, heteroarilo, arilalquilo y heteroarilalquilo están opcionalmente sustituidos con uno, dos o tres sustituyentes seleccionados independientemente entre halo, alquilo C_{1-6} , haloalquilo C_{1-6} , cicloalquilo C_{3-6} , haloalcoxi, $-SR^9$, $-SR^{12}$, $-SOR^9$, $-SOR^{12}$, $-SO_2R^9$, $-SO_2R^{12}$, $-SO_2CH(R^{12})(R^{11})$, $-OR^{12}$, $-N(R^{10})(R^{11})$, ciano, o arilo que está opcionalmente sustituido con $-SO_2R^{12}$;

cada D es independientemente alquilo C_{1-3} , alqueno C_{2-3} , alquilo C_{2-3} , arilo, heteroarilo, cicloalquilo C_{3-8} o heterociclilo donde cada uno de dichos grupos arilo, heteroarilo, cicloalquilo y heterociclilo, que pueden ser monocíclicos o bicíclicos, están opcionalmente sustituidos sobre el átomo de carbono o el heteroátomo con de uno a cinco sustituyentes seleccionados independientemente entre alquilo C_{1-6} , haloalquilo, halo, ceto, alcoxi, $-SR^9$, $-SR^{12}$, $-OR^9$, $-OR^{12}$, $-N(R^{12})_2$, $-SO_2R^9$, o $-SO_2R^{10}$;

R^7 es hidrógeno, alquilo C_{1-6} , $-SO_2R^{12}$, $-SO(R^{12})$, $-OSO_2R^{10}$, $-SOMN(R^c)(R^d)$, $-SOMCH(R^{10})(R^{11})$, o $-SOM(R^c)(R^d)$;

R^8 es hidrógeno o alquilo C_{1-6} ;

o R^4 y R^8 pueden tomarse junto con cualquiera de los átomos a los que pueden estar unidos para formar un sistema de anillos heterociclilo de 4-10 miembros donde dicho sistema de anillos, que pueden ser monocíclicos o bicíclicos, está opcionalmente sustituido con uno o dos sustituyentes seleccionados independientemente entre alquilo C_{1-6} , halo, hidroxialquilo, hidroxilo, ceto, $-OR^{10}$, $-SR^{10}$ o $-N(R^{10})_2$;

R^9 se selecciona entre el grupo compuesto por hidrógeno, arilo, aril-alquilo (C_{1-4}), heteroarilo, heteroaril-alquilo (C_{1-4}), cicloalquilo C_{3-8} , cicloalquil C_{3-8} -alquilo (C_{1-4}) y heterociclil-alquilo (C_{1-4}) donde dichos grupos pueden estar opcionalmente sustituidos con uno, dos o

tres sustituyentes seleccionados independientemente entre halo, alcoxi o $-\text{SO}_2\text{R}^{12}$;

R^{10} es hidrógeno o alquilo C_{1-6}

R^{11} es hidrógeno o alquilo C_{1-6} ;

R^{12} es hidrógeno o alquilo C_{1-6} que está opcionalmente sustituido con uno, dos o tres sustituyentes seleccionados independientemente entre halo, alcoxi, ciano, $-\text{NR}^{10}$ o $-\text{SR}^{10}$;

R^a es hidrógeno, alquilo C_{1-6} , (alquil C_{1-6})arilo, (alquil C_{1-6})hidroxilo, -O(alquil C_{1-6}), hidroxilo, halo, arilo, heteroarilo, cicloalquilo C_{3-8} , heterociclilo, donde dicho alquilo, arilo, heteroarilo, cicloalquilo C_{3-8} y heterociclilo pueden estar opcionalmente sustituidos sobre el carbono o el heteroátomo con uno, dos o tres sustituyentes seleccionados independientemente entre alquilo C_{1-6} o halo;

R^b es hidrógeno, alquilo C_{1-6} , (alquil C_{1-6})arilo, (alquil C_{1-6})hidroxilo, alcoxilo, hidroxilo, halo, arilo, heteroarilo, cicloalquilo C_{3-8} o heterociclilo, donde dicho alquilo, arilo, heteroarilo, cicloalquilo C_{3-8} y heterociclilo pueden estar opcionalmente sustituidos sobre el carbono o el heteroátomo con uno, dos o tres sustituyentes seleccionados independientemente entre alquilo C_{1-6} o halo;

o R^a y R^b pueden tomarse junto con el átomo de carbono al que están unidos o los que están entre ellos para formar un anillo cicloalquilo C_{3-8} o un anillo C_{3-8} heterociclilo donde dicho sistema de anillos de 3-8 miembros puede estar opcionalmente sustituido con uno o dos sustituyentes seleccionados independientemente entre alquilo C_{1-6} y halo;

R^c es hidrógeno o alquilo C_{1-6} que está opcionalmente sustituido con uno, dos o tres sustituyentes seleccionados independientemente entre halo o $-\text{OR}^9$;

R^d es hidrógeno o alquilo C_{1-6} que está opcionalmente sustituido con uno, dos o tres sustituyentes seleccionados independientemente entre halo o $-\text{OR}^9$;

o R^c y R^d pueden tomarse junto con el átomo de nitrógeno al que están unidos o los que están entre ellos para formar un anillo C_{3-8} heterociclilo que está opcionalmente sustituido con uno o dos sustituyentes seleccionados independientemente entre alquilo C_{1-6} , halo hidroxialquilo, hidroxilo, alcoxi o ceto;

n se selecciona independientemente entre un número entero de uno a tres;

cada m se selecciona independientemente entre un número entero de cero a dos;

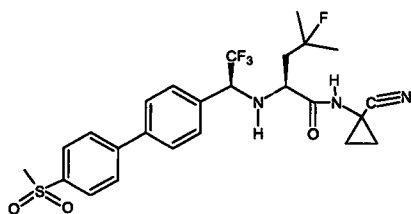
donde "arilo" es cualquier anillo de carbonos monocíclico o bicíclico estable de hasta 12 átomos en cada anillo, donde a menos un anillo es aromático; y "heteroarilo" representa un anillo monocíclico, bicíclico o tricíclico de hasta 10 átomos en cada anillo, donde al menos un anillo es aromático y contiene de 1 a 4 heteroátomos seleccionados entre el grupo compuesto por O, N y S.

y las sales estereoisómeros y derivados de N-óxido farmacéuticamente aceptables de los mismos."

Me permito, además, señalar que las equivalencias de todos los sustituyentes del compuesto de fórmula 1 se encuentran plenamente sustentados en la descripción, donde se muestran 38 ejemplos de síntesis y se consideran específicamente más de 450 compuestos (al igual que en la reivindicación 9).

La persona versada en la materia al referirse a estos compuestos podría comprender la diversidad de posibles sustituyentes que conforman los compuestos que se están reclamando, y reconocer que no es posible limitar más el significado de dichos sustituyentes sin excluir algunos de los compuestos específicamente señalados.

Como ejemplo de esto me permito mostrar las equivalencias de los sustituyentes correspondientes a algunos de los compuestos específicos revelados en la solicitud en estudio:



Ejemplo 15 (odanacatib)

R^1 y R^2 = anillo cicloalquilo C_3

R^3 = H

R^4 = alquilo C_4 sustituido halo 1

R^5 = H

R^6 = halo alquilo C_1

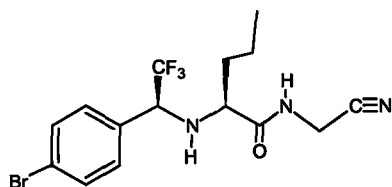
R^8 = H

n = 2

D = arilo

R^7 = $-SO_2R^{12}$

R^{12} = alquilo C_1



Ejemplo 23

R^1 y $R^2 = H$

$R^3 = H$

$R^4 =$ alquilo C_3

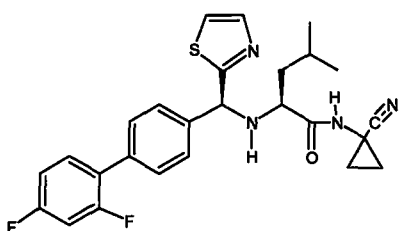
$R^5 = H$

$R^6 =$ halo alquilo C_1

$R^8 = H$

$n = 1$

$D =$ arilo sustituido halo



Ejemplo 36

R^1 y $R^2 =$ anillo cicloalquilo C_3

$R^3 = H$

$R^4 =$ alquilo C_4

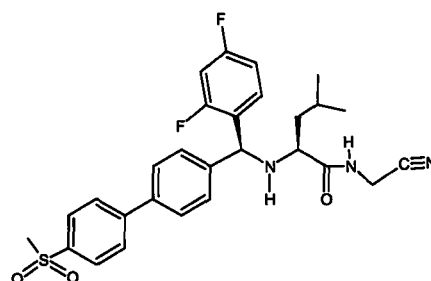
$R^5 = H$

$R^6 =$ heteroarilo de cinco miembros
con 2 heteroátomos S y N

$R^8 = H$

$n = 2$

$D =$ arilo y arilo di halo sustituido



Ejemplo 20

R^1 y $R^2 = H$

$R^3 = H$

$R^4 =$ alquilo C_4

$R^5 = H$

$R^6 =$ arilo disustituido halo

$R^7 =$

$R^8 = H$

$n = 2$

$D =$ arilo

$R^7 = -SO_2R^{12}$

$R^{12} =$ alquilo C_1

Así los significados de todos los sustituyentes considerados en la reivindicación uno se encuentran sustentados en la descripción y son claros al definir el objeto de la presente invención.

- Reivindicaciones 2 a 4. Estas reivindicaciones no han sido modificadas y corresponden a las originalmente presentadas.
- Reivindicación 5. Al igual que en la reivindicación 1, con el fin de clarificar los compuestos reclamados, se introduce el significado de los términos "arilo" y "heteroarilo".
- Reivindicaciones 6 y 7. Estas reivindicaciones no han sido modificadas y corresponden a las originalmente presentadas.
- Reivindicación 8. Al igual que en las reivindicaciones 1 y 5, se introduce el significado de los términos "arilo" y "heteroarilo".
- Reivindicaciones 9. Se modifica el encabezado de esta reivindicación haciéndola independiente. De esta forma el encabezado se lee "*Un compuesto seleccionado entre:*". Todos los compuestos aquí relacionados se encuentran consignados en la descripción (páginas 32 a 62).
- Reivindicaciones 10 a 13. Estas reivindicaciones no han sido modificadas y corresponden a las originalmente presentadas.
- Reivindicación 14. Acatando la sugerencia del señor examinador se introduce el término "*en una cantidad terapéuticamente eficaz*", al referirse al compuesto de la reivindicación 1.
- Reivindicación 15. Teniendo en consideración la objeción de claridad expuesta por el Señor Examinador con respecto a esta reivindicación, es deseo del solicitante eliminarla del capítulo reivindicatorio.
- Reivindicación 16. Tomando en cuenta la objeción expuesta por El Señor Examinador con respecto a esta reivindicación por referirse a un

procedimiento que no detalla pasos, es deseo del solicitante **eliminarla del capítulo reivindicatorio.**

- **Reivindicaciones 17 a 21.** Acatando la objeción expuesta por el Señor Examinador con respecto a estas reivindicaciones, por considerar métodos terapéuticos, es deseo del solicitante **eliminarlas del capítulo reivindicatorio.**
- **Reivindicación 22 (Nueva reivindicación 15).** Considerando la objeción del Señor Examinador con respecto a la claridad de esta reivindicación, se han especificado los tipos de agentes que pueden acompañar al compuesto de la reivindicación 1 en la formulación farmacéutica. Todos los compuestos aquí relacionados son mostrados en la descripción como los principios activos preferidos en las composiciones farmacéuticas que contienen los compuestos de la reivindicación 1 (Páginas 69 a 80).
- **Reivindicaciones 23 y 24.** Acatando la objeción expuesta por el Señor Examinador con respecto a estas reivindicaciones, por considerar métodos terapéuticos, es deseo del solicitante **eliminarlas del capítulo reivindicatorio.**
- **Reivindicación 25.** Tomando en cuenta la objeción expuesta por El Señor Examinador con respecto a la claridad de esta reivindicación, es deseo del solicitante **eliminarla del capítulo reivindicatorio.**
- **Reivindicaciones 26 a 28.** Considerando la objeción expuesta por el Señor Examinador con respecto a estas reivindicaciones, por considerar métodos terapéuticos, es deseo del solicitante **eliminarlas del capítulo reivindicatorio.**

Me permito señalar que las modificaciones realizadas en el capítulo reivindicatorio clarifican completamente los compuestos reclamados en la solicitud en estudio, encontrándose por tanto dicho capítulo dentro de las consideraciones del Artículo 30 de la Decisión Andina 486 de 2000.

4. Unidad de Invención.

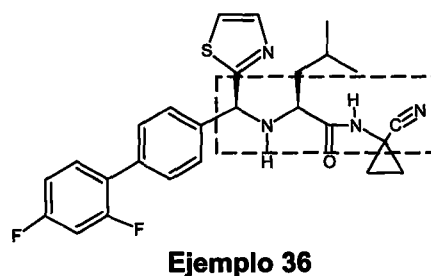
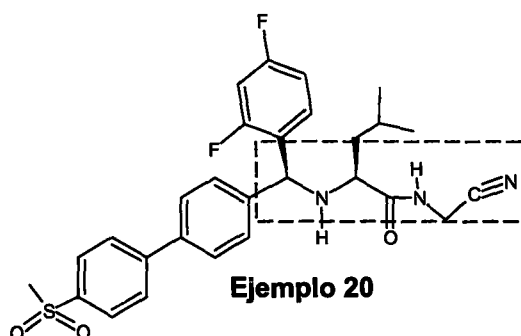
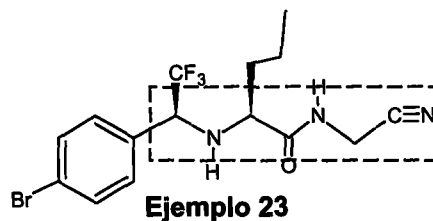
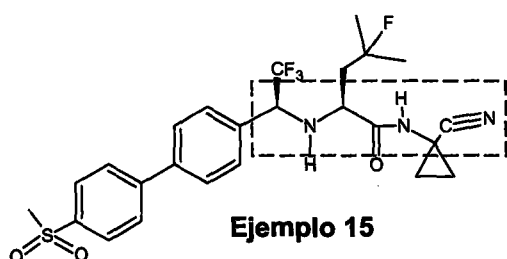
Por otra parte el señor examinador asegura que la presente solicitud carece de unidad de invención, señalando que no hay una característica técnica común entre los compuestos reclamados dado que *"cada terminación en el radical R⁷ (grupos 1-8) le confiere al compuesto diferentes características y propiedades químicas, modos de acción y efectos diferentes"*.

Sobre este particular me permito señalar que todos los compuestos considerados en la presente solicitud comparten una estructura básica común, que hace que tengan una relación estructural que los convierte en una sola unidad de invención.

Este hecho es más evidente a la luz de las modificaciones realizadas en la reivindicación 1, donde se limita el número de posibles significados de R⁷, **haciendo que este grupo no pueda ser más importante que el esqueleto central.**

Me permito presentar como muestra de este hecho las estructuras de los ejemplos 15, 20, 23 y 36 antes mencionados, donde es evidente que **la estructura principal es un derivado de un α -aminoácido que tiene un nitrógeno sobre el carbonilo.** Dicho nitrógeno se encuentra a su vez unido a un carbono que soporta un grupo ciano. El nitrógeno α al carbonilo se une a un carbono que sirve de puente a un grupo que puede ser alifático o al menos un anillo aromático.

En la figura se resalta la estructura común que presentan todos los compuestos considerados en la solicitud en estudio:



Me permito señalar que todos los compuestos considerados en la solicitud en estudio comparten este mismo "esqueleto" central en su estructura, y que **los sustituyentes adicionales, aunque presentan influencia en la actividad del compuesto, no son estructuralmente más importantes que la unidad central común.** De esta forma, todos los compuestos revelados en la presente solicitud pueden considerarse derivados de una misma unidad estructural.

Esto es igualmente visible en los documentos adicionales citados anteriormente y que se adjuntan con este memorial, donde se expone de forma clara cómo **todos los compuestos mantienen una relación estructural,** y que **a pesar de los cambios de sustituyentes su actividad como inhibidores de catepsinas, dicha estructura es semejante.**

Teniendo en consideración lo anteriormente expuesto, me permito manifestar que todos los compuestos de la invención comparten una misma estructura central y, por lo tanto, **se constituyen en una sola unidad de invención.**

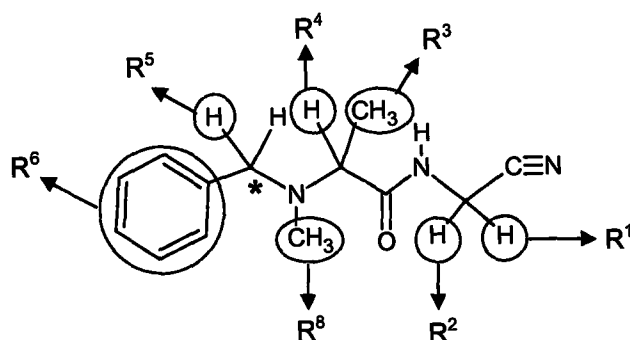
5. **Novedad.**

Finalmente, el Señor Examinador objeta la novedad de la solicitud en estudio frente a las enseñanzas del documento **CO-03104808 (D1)**, que revela compuestos que tienen actividad como inhibidores de catepsina S. Con respecto a dicho documento el Señor Examinador declara que compuestos específicos considerados en D1 *"son iguales a compuestos de la presente solicitud, tras hacer los reemplazos respectivos de sustituyentes"*. Además, el Señor Examinador señala *"Si se mira la reivindicación 1 de dicha anterioridad, se puede apreciar que puede haber más compuestos idénticos a compuestos de la actual solicitud, al hacer los correspondientes reemplazos de grupos sustituyentes."*

A ese respecto me permito manifestar que, teniendo en consideración los significados de todos los sustituyentes incluidos en la formula 1 de la solicitud en estudio, **no existe la posibilidad que compuestos revelados en D1 se encuentren incluidos dentro de los compuestos de la presente solicitud,** como me permito mostrar a continuación:

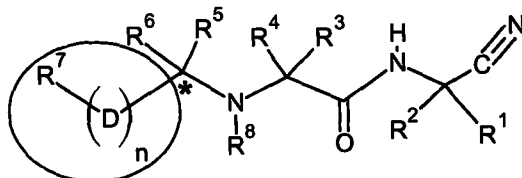
Tenga en cuenta que **para que los compuestos considerados en D1 pudieran estar dentro de los reclamados en la presente solicitud se requeriría que no existiera un grupo D en la molécula, es decir que n fuera igual a cero.** Como se mencionó anteriormente, en la reivindicación 1 el valor de n fue erróneamente consignado como *"entre un número entero de cero a tres"*, y no *"entre un número entero de uno a tres"* como se encuentra escrito en la reivindicación 1 de la solicitud internacional (PCT/US03/06147, página 230). De esta forma, **teniendo en cuenta la corrección realizada en la reivindicación 1 con respecto al significado de n , no existe posibilidad que ninguno de los compuestos considerados en D1 corresponda a algún compuesto de los revelados en la solicitud en estudio.**

Como ejemplo de lo anteriormente expuesto, me permito puntualizar en una de las moléculas que fueron señaladas por el Señor Examinador como iguales a algunas de las consideradas en la presente solicitud:



Compuesto de D1

En la muestra las figura se



Formula 1 presente solicitud

correspondencias de los sustituyentes presentes en el compuesto considerado en el documento D1 con los sustituyentes de la fórmula 1 de la solicitud en estudio. Como lo señala el Señor Examinador los posibles significados de estos sustituyentes podrían construir la molécula mostrada. Sin embargo, la única posibilidad para que dicho compuesto de D1 fuera parte de los revelados en la solicitud en estudio es que no exista ningún grupo D ni R⁷ en la molécula. Como se mencionó anteriormente, al corregir el error material cometido en la reivindicación 1, es claro que el valor de n no puede ser cero, por lo cual tendría que existir al menos uno grupo D en la molécula. De esta forma, el carbono adyacente al nitrógeno amínico (marcado en la figura con *) necesariamente es terciario (o cuaternario), y no secundario como en el compuesto correspondiente a D1.

Esta diferencia en el número de sustituyentes sobre el carbono adyacente al nitrógeno amínico excluye completamente la posibilidad de que los compuestos revelados en la solicitud en estudio puedan estar incluidos dentro de los compuestos considerados en D1.

El análisis anterior es válido para todos los compuestos considerados en D1 y señalados como equivalentes a los de la presente solicitud por el Señor Examinador. De esta forma dichos compuestos de D1 son diferentes con respecto a los considerados en la solicitud en estudio.

Considerando lo anteriormente expuesto, me permito resaltar que los compuestos considerados en la presente solicitud **no pueden estar incluidos en el citado documento D1, siendo por lo tanto la presente solicitud novedosa frente a las enseñanzas de dicho documento D1, y cumpliendo con los requisitos exigidos por el Artículo 16 de la Decisión Andina 486 de 2000.**

6. **Conclusión.**

Finalmente, me permito manifestar que los argumentos expuestos con respecto a la claridad del capítulo descriptivo, junto con las modificaciones realizadas sobre el capítulo reivindicatorio, han mostrado que **la presente solicitud es clara y suficiente para describir la invención reclamada.**

De igual forma, los argumentos técnicos discutidos mostraron que todos los compuestos considerados en la presente solicitud incluyen una estructura química común, haciendo que se constituyan en **una sola unidad de invención.**

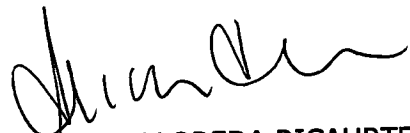
Sumado a lo anterior, los argumentos expuestos con respecto al documento D1 junto con dichos cambios en el capítulo reivindicatorio, excluyen de manera absoluta la posibilidad que los compuestos materia de la presente invención se encuentren incluidos en aquellos revelados en el documento D1, demostrando **la novedad de la presente invención frente al estado del arte más próximo.**

7. Anexo me permito presentar:

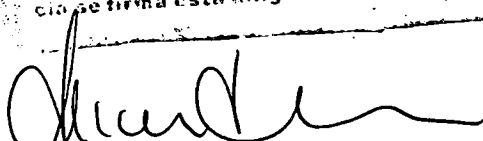
- (a) Recibo número 08-17.033, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de las modificaciones mencionadas;
- (b) Copia del nuevo capítulo reivindicatorio, en el cual se han realizado las modificaciones expuestas anteriormente; y
- (c) Copia de los documentos *Bioorganic & Medicinal Chemistry Letters* 15 (2005) 4741-4744, *Bioorganic & Medicinal Chemistry Letters* 16 (2006) 1985-1989, *ChemBioChem*, 2006, 7, 1525-1535, *Bioorganic & Medicinal Chemistry Letters* 18 (2008) 923-928, *Clinical pharmacology & Therapeutics*, Vol. 83, enero 2008, 172-176, referenciados en el texto.

8. En vista de todo lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las modificaciones y los argumentos presentados y conceder el privilegio de patente solicitado.

Señor Superintendente,


ALICIA LLORED A RICAURTE
T.P. 53.215

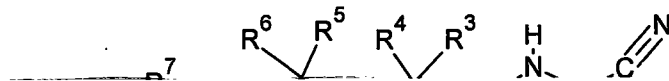
NOTARIA S E X T A DE BOGOT A
RECONOCIMIENTO Y PRESENTACION PERSONAL
Bogotá, D.C. **12 MAR 2008**
Ante mí **PABLO MENDEZ SERRAÑOS**
NOTARIO SEXTO (E) DEL CIRCULO DE BOGOT A
Identificación: **Alicia Lloreda Ricaurte**
C.C. # 39.690.713
T.P. # 53.215
y declaro (ellos) que lo (s) firma (s) que aparece (n) en el presente documento es (son) la (s) suya (s) y que el contenido del mismo es cierto. En constancia se firma esta diligencia.


ALICIA LLORED A RICAURTE
C.C. No. 39.690.713 de Usaquén



REIVINDICACIONES

1. La presente invención se refiere a compuestos de la siguiente formula química:



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 900176089-2

RECIBO OFICIAL DE CAJA : 08 - 17,033
FECHA : FEBRERO 27 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	Nº. PAGO	FECHA PGO	Vr. PAGO
LLOREDA Y CIA S. A	CONSIGNACION	BANCO DE BOGOTA	062754387	769742	27/02/2008	8,623,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEMAS COMERCIALES	103,000.00
		TOTAL :	\$ 103,000.00

SON: CIENTO TRES MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No.

R^6 es arilo, heteroarilo, haloalquilo C_{1-6} , arilalquilo o heteroarilalquilo, donde dichos grupos arilo, heteroarilo, arilalquilo y heteroarilalquilo están opcionalmente sustituidos con uno, dos o tres sustituyentes seleccionados independientemente entre halo, alquilo C_{1-6} , haloalquilo C_{1-6} , cicloalquilo C_{3-6} , haloalcoxi, $-SR^9$, $-SR^{12}$, $-SOR^9$, $-SOR^{12}$, $-SO_2R^9$, $-SO_2R^{12}$, $-SO_2CH(R^{12})(R^{11})$, $-OR^{12}$, $-N(R^{10})(R^{11})$, ciano, o arilo que está opcionalmente sustituido con $-SO_2R^{12}$;

cada D es independientemente alquilo C_{1-3} , alqueno C_{2-3} , alquino C_{2-3} , arilo, heteroarilo, cicloalquilo C_{3-8} o heterociclilo donde cada uno de dichos grupos arilo, heteroarilo, cicloalquilo y heterociclilo, que pueden ser monocíclicos o bicíclicos, están opcionalmente sustituidos sobre el átomo de carbono o el heteroátomo con de uno a cinco sustituyentes seleccionados independientemente entre alquilo C_{1-6} , haloalquilo, halo, ceto, alcoxi, $-SR^9$, $-SR^{12}$, $-OR^9$, $-OR^{12}$, $-N(R^{12})_2$, $-SO_2R^9$, o $-SO_2R^{10}$;

R^7 es hidrógeno, alquilo C_{1-6} , $-SO_2R^{12}$, $-SO(R^{12})$, $-OSO_2R^{10}$, $-SOMN(R^c)(R^d)$, $-SOMCH(R^{10})(R^{11})$, o $-SOM(R^c)(R^d)$;

R^8 es hidrógeno o alquilo C_{1-6} ;

o R^4 y R^8 pueden tomarse junto con cualquiera de los átomos a los que pueden estar unidos para formar un sistema de anillos heterociclilo de 4-10 miembros donde dicho sistema de anillos, que pueden ser monocíclicos o bicíclicos, está opcionalmente sustituido con uno o dos sustituyentes seleccionados independientemente entre alquilo C_{1-6} , halo, hidroxialquilo, hidroxilo, ceto, $-OR^{10}$, $-SR^{10}$ o $-N(R^{10})_2$;

R^9 se selecciona entre el grupo compuesto por hidrógeno, arilo, aril-alquilo (C_{1-4}), heteroarilo, heteroaril-alquilo (C_{1-4}), cicloalquilo C_{3-8} , cicloalquil C_{3-8} -alquilo (C_{1-4}) y heterocicilil-alquilo (C_{1-4}) donde dichos grupos pueden estar opcionalmente sustituidos con uno, dos o tres sustituyentes seleccionados independientemente entre halo, alcoxi o $-SO_2R^{12}$;

R^{10} es hidrógeno o alquilo C_{1-6}

R^{11} es hidrógeno o alquilo C_{1-6} ;

R^{12} es hidrógeno o alquilo C_{1-6} que está opcionalmente sustituido con uno, dos o tres sustituyentes seleccionados independientemente entre halo, alcoxi, ciano, $-NR^{10}$ o $-SR^{10}$;

R^a es hidrógeno, alquilo C_{1-6} , (alquil C_{1-6})arilo, (alquil C_{1-6})hidroxilo, $-O$ (alquil C_{1-6}), hidroxilo, halo, arilo, heteroarilo, cicloalquilo C_{3-8} , heterociclilo, donde dicho alquilo, arilo, heteroarilo, cicloalquilo C_{3-8} y heterociclilo pueden estar

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394

opcionalmente sustituidos sobre el carbono o el heteroátomo[?] con uno, dos o tres sustituyentes seleccionados independientemente entre alquilo C₁₋₆ o halo;

R^b es hidrógeno, alquilo C₁₋₆, (alquil C₁₋₆)arilo, (alquil C₁₋₆)hidroxilo, alcoxilo,
5 hidroxilo, halo, arilo, heteroarilo, cicloalquilo C₃₋₈ o heterociclilo, donde dicho alquilo, arilo, heteroarilo, cicloalquilo C₃₋₈ y heterociclilo pueden estar opcionalmente sustituidos sobre el carbono o el heteroátomo[?] con uno, dos o tres sustituyentes seleccionados independientemente entre alquilo C₁₋₆ o halo;

o R^a y R^b pueden tomarse junto con el átomo de carbono al que están unidos o
10 los que están entre ellos para formar un anillo cicloalquilo C₃₋₈ o un anillo C₃₋₈ heterociclilo donde dicho sistema de anillos de 3-8 miembros puede estar opcionalmente [?] sustituido con uno o dos sustituyentes seleccionados independientemente entre alquilo C₁₋₆ y halo;

R^c es hidrógeno o alquilo C₁₋₆ que está opcionalmente sustituido con uno, dos
15 o tres sustituyentes seleccionados independientemente entre halo o -OR⁹;

R^d es hidrógeno o alquilo C₁₋₆ que está opcionalmente sustituido con uno, dos o tres sustituyentes seleccionados independientemente entre halo o -OR⁹;

o R^c y R^d pueden tomarse junto con el átomo de nitrógeno al que están unidos o los que están entre ellos para formar un anillo C₃₋₈ [?] heterociclilo que está
20 opcionalmente sustituido con uno o dos sustituyentes seleccionados independientemente entre alquilo C₁₋₆, halo hidroxialquilo, hidroxi, alcoxi o ceto;

n se selecciona independientemente entre un número entero de uno a tres;

cada m se selecciona independientemente entre un número entero de cero a dos;

25 donde "arilo" es cualquier anillo de carbonos monocíclico o bicíclico estable de [?] hasta 12 átomos en cada anillo, donde a menos un anillo es aromático; y "heteroarilo" representa un anillo monocíclico, bicíclico o tricíclico de hasta [?] 10 átomos en cada anillo, donde al menos un anillo es aromático y contiene de 1 a 4 heteroátomos seleccionados entre el grupo compuesto por O, N y S.

30 y las sales estereoisómeros y derivados de N-óxido farmacéuticamente aceptables de los mismos.

2. El compuesto de la reivindicación 1 donde R¹ es hidrógeno y R² es hidrógeno, o R¹ y R² pueden tomarse junto con el átomo de carbono al que están
35 unidos para formar un anillo de cicloalquilo C₃₋₈ estando dicho sistema de anillo

opcionalmente sustituido con uno o dos sustituyentes seleccionados entre alquilo C₁₋₆ o halo, y las sales, estereoisómeros y derivados de N-óxido farmacéuticamente aceptables del mismo.

- 5 3. El compuesto de la reivindicación 2 donde R³ es alquilo C₁₋₆ que está
opcionalmente sustituido con de uno a seis halo y R⁴ es hidrógeno, y las sales,
estereoisómeros y derivados de N-óxido farmacéuticamente aceptables del mismo.
- 10 4. El compuesto de la reivindicación 3 donde R³ es n-propil, isobutilo, 2-
fluoro-2-metilpropilo, 2-trifluorometilpropilo, 3-fluoro-2-(2-fluorometil)propilo, 2,2-
difluoroetilo, 2,2-difluoropropilo, 3,3,3-trifluoropropilo, o 2,2-dicloroetilo y R⁴ es
hidrógeno; y las sales, estereoisómeros y derivados de N-óxido farmacéuticamente
aceptables del mismo.
- 15 5. El compuesto de la reivindicación 3 donde R⁵ es hidrógeno y R⁶ es arilo
o heteroarilo, donde “arilo” es cualquier anillo de carbonos monocíclico o bicíclico
estable de hasta 12 átomos en cada anillo, donde a menos un anillo es aromático; y
“heteroarilo” representa un anillo monocíclico, bicíclico o tricíclico de hasta 10
20 átomos en cada anillo, donde al menos un anillo es aromático y contiene de 1 a 4
heteroátomos seleccionados entre el grupo compuesto por O, N y S, estando dicho
arilo o heteroarilo opcionalmente sustituido con uno, dos o tres sustituyentes
seleccionados entre halo o -SO₂R¹²; las sales, estereoisómeros y derivados de N-
óxido farmacéuticamente aceptables del mismo.
- 25 6. El compuesto de la reivindicación 3 donde R⁵ es hidrógeno y R⁶ es alquilo
C₁₋₆ sustituido con 1-6 halo; las sales, estereoisómeros y derivados de N-óxido
farmacéuticamente aceptables del mismo.
- 30 7. El compuesto de la reivindicación 6 donde R⁵ es hidrógeno y R⁶ es
trifluorometilo o 3,3,3,2,2-pentafluoroetilo; las sales, estereoisómeros y derivados de
N-óxido farmacéuticamente aceptables del mismo.
- 35 8. El compuesto de la reivindicación 6 donde n es dos, cada D es
independientemente arilo o heteroarilo y R⁷ es -SO_mCH(R¹⁰)(R¹¹) o
-SO_m(R^c)(R^d), cada R^c y R^d es independientemente hidrógeno o alquilo C₁₋₆; donde

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“arilo” es cualquier anillo de carbonos monocíclico o bicíclico estable de hasta 12 átomos en cada anillo, donde a menos un anillo es aromático; y “heteroarilo” representa un anillo monocíclico, bicíclico o tricíclico de hasta 10 átomos en cada anillo, donde al menos un anillo es aromático y contiene de 1 a 4 heteroátomos seleccionados entre el grupo compuesto por O, N y S; y las sales, estereoisómeros y derivados de N-óxido farmacéuticamente aceptables del mismo.

9. Un compuesto seleccionado entre:

- 10 • N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(cianometil)-N²-(2,2,2-trifluoro-1-feniletal)-L-leucinamida;
- N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-fluoro-3-metilfenil)etil]-L-leucinamida;
- 15 • N¹-(cianometil)-N²-[(1R)-2,2,2-trifluoro-1-(4-piridin-3-ilfenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-piridin-3-ilfenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1R)-2,2,2-trifluoro-1-(4-piridin-4-ilfenil)etil]-L-leucinamida;
- 20 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-piridin-4-ilfenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1R)-2,2,2-trifluoro-1-(4-{[-(2-fluoroetil)piperazin-1-il]carbonil}fenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-{[4-(2-fluoroetil)piperazin-1-il]carbonil}fenil)etil]-L-leucinamida;
- 25 • N²-[1-(1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4-(3-hidroxi-3-metilbut-1-inil)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(3-hidroxi-3-metilbutil)fenil]etil}-L-leucinamida;
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- N²-[(1S)-1-(4-bromofenil)-2,2,3,3,3-pentafluoropropil]-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,3,3,3-pentafluoro-1-(4-piridin-4-ilfenil)propil]-L-leucinamida;
- 5 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4'-fluoro-1,1'-bifenil-4-il)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-{4-[(1E)-3-hidroxi-3-metilbut-1-enil]fenil}etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,3,3,3-pentafluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]propil]-L-leucinamida;
- 10 • N¹-(cianometil)-N²-[(1S)-2,2,3,3,3-pentafluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]propil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-[4-(1-oxidopiridin-3-il)fenil]etil]-L-leucinamida;
- 15 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-[4-(morfolin-4-ilocarbonil)fenil]etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-{[metoxi(metil)amino]carbonil}fenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-tien-3-ilfenil)etil]-L-leucinamida;
- 20 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-[4-(4-metilpiridin-2-il)fenil]etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-[4-(5-metilpiridin-2-il)fenil]etil]-L-leucinamida;
- 25 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(2'-fluoro-1,1'-bifenil-4-il)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-1-[4-(3,5-dimetilisoxazol-4-il)fenil]-2,2,2-trifluoroetil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-[4'-(hidroximetil)-1,1'-bifenil-4-il]etil]-L-leucinamida;
- 30 • N²-[(1S)-1-(4'-ciano-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-1-(3',4'-difluoro-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-L-leucinamida;
- 35 -éster metílico del ácido 4'-{1-[1-(Cianometil-carbamoil)-3-metil-butilamino]-

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- 2,2,2-trifluoro-etil}-bifenil-2-carboxílico;
 • éster metílico del ácido 4'-{1-[1-(Cianometil-carbamoil)-3-metil-butilamino]-
 2,2,2-trifluoro-etil}-bifenil-3-carboxílico;
 • N¹-(cianometil)-N²-[(1S)-1-(3',4'-dimetoxi-1,1'-bifenil-4-il)-2,2,2-
 5 trifluoroetil]-L-leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[2'-(trifluorometil)-1,1'-bifenil-4-
 il]etil}-L-leucinamida;
 • N¹-(cianometil)-N²-[(1S)-1-(3',4'-dicloro-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-
 L-leucinamida;
 10 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(3'-formil-1,1'-bifenil-4-il)etil]-L-
 leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-oxo-2,3-dihidro-1,3-
 benzotiazol-6-il)fenil]etil}-L-leucinamida;
 • N²-{(1S)-1-[4-(5-bromopiridin-3-il)fenil]-2,2,2-trifluoroetil}-N¹-(cianometil)-
 15 L-leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(trifluorometoxi)-1,1'-bifenil-4-
 il]etil}-L-leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1H-indol-4-il)fenil]etil}-L-
 leucinamida;
 20 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-pirimidin-5-ilfenil)etil]-L-
 leucinamida;
 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-quinolin-3-ilfenil)etil]-L-
 leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1,3-tiazol-2-il)fenil]etil}-L-
 25 leucinamida;
 • éster metílico del ácido 4'-{1-[1-(cianometil-carbamoil)-3-metil-butilamino]-
 2,2,2-trifluoro-etil}-bifenil-4-carboxílico;
 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-pirimidin-2-ilfenil)etil]-L-
 leucinamida;
 30 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(3-metilpiridin-2-il)fenil]etil}-
 L-leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(3-furil)fenil]etil}-L-
 leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-[3-(trifluorometil)piridin-2-
 35 il]fenil]etil}-L-leucinamida;

- . N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4-[4-(trifluorometil)piridin-2-il]fenil}etil)-L-leucinamida;
- . N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4-[5-(trifluorometil)piridin-2-il]fenil}etil)-L-leucinamida;
- 5 . N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(3'-metoxi-1,1'-bifenil-4-il)etil]-L-leucinamida;
- . N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(3'-metoxi-1,1'-bifenil-4-il)etil]-L-leucinamida;
- . N²-{(1S)-1-[4'-(acetilamino)-3'-fluoro-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-
- 10 N¹-(cianometil)-L-leucinamida;
- . N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(3-metiltien-2-il)fenil]etil}-L-leucinamida;
- . N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(3'-fluoro-1,1'-bifenil-4-il)etil]-L-leucinamida;
- 15 . N²-{(1S)-1-[4-(5-acetiltien-2-il)fenil]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
- . N²-[(1S)-1-(3'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- . N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[3'-(trifluorometil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 20 . N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(5'-fluoro-2'-metoxi-1,1'-bifenil-4-il)etil]-L-leucinamida;
- . N¹-(cianometil)-N²-[(1S)-1-(3',5'-difluoro-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-L-leucinamida;
- 25 . N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(2',3',5'-trifluoro-1,1'-bifenil-4-il)etil]-L-leucinamida;
- . ácido 3-(4'-{1-[1-(Cianometil-carbamoil)-3-metil-butilamino]-2,2,2-trifluoroetil}-bifenil-3-il)-acrílico;
- . N²-{(1S)-1-[4-(9-antril)fenil]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-
- 30 leucinamida;
- . N²-[(1S)-1-(4'-benzoil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- . N²-[(1S)-1-(3'-acetil-4'-hidroxi-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- 35 . N¹-(cianometil)-N²-{(1S)-1-[2'-(cianometil)-1,1'-bifenil-4-il]-2,2,2-

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- trifluoroetil}-L-leucinamida;
- N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4'-(metilsulfinil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 5 L-leucinamida;
- N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-morfolin-4-ilfenil)etil]-L-leucinamida;
 - N¹-(cianometil)-N²-{(1R)-2,2,2-trifluoro-1-[4-(6-metilpiridin-3-il)fenil]etil}-L-leucinamida;
- 10 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metilpiridin-3-il)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(5-fenilotien-2-il)etil]-L-leucinamida;
 - N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-quinolin-8-ilfenil)etil]-L-leucinamida;
- 15 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-piridin-2-ilfenil)etil]-L-leucinamida;
- N²-{1-[4'-(aminosulfonil)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
 - N²-{(1S)-1-[4'-(aminosulfonil)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
- 20 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 25 • N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-{(1R)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(morfolin-4-ilosulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 30 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(isopropilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N²-{(1S)-1-[4'-(aminosulfonil)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
 - N²-{(1S)-1-[4'-(acetilamino)sulfonil]-1,1'-bifenil-4-il}-2,2,2-trifluoroetil)-N¹-(cianometil)-L-leucinamida;
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- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[2'-metil-4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N²-[1-(5-bromotien-2-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- N²-[1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- 5 • éster *terc*-butílico del ácido 4-(4'-{1-[1-(Cianometil-carbamoi)-3-metil-butilamino]-2,2,2-trifluoro-etil}-bifenil-4-il)-piperazina-1-carboxílico;
- N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4'-piperazin-1-il-1,1'-bifenil-4-il)etil]-L-leucinamida;
- N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4'-[4-(2-hidroxi-etil)piperazin-1-il]-1,1'-bifenil-4-il}etil)-L-leucinamida;
- 10 • N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4'-[4-(2-hidroxi-2-metilpropil)piperazin-1-il]-1,1'-bifenil-4-il}etil)-L-leucinamida;
- N¹-(cianometil)-N²-(1-{4-[(dimetilamino)carbonil]fenil}-2,2,2-trifluoroetil)-L-leucinamida;
- 15 • N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4'-piperazin-1-il-1,1'-bifenil-4-il)etil]-L-leucinamida;
- N¹-(cianometil)-N²-(1-{4-[(ciclopropilamino)carbonil]fenil}-2,2,2-trifluoroetil)-L-leucinamida;
- ácido 4-{1-[1-(Cianometil-carbamoi)-3-metil-butilamino]-2,2,2-trifluoro-etil}-benzoico;
- 20 • N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4'-[4-(2-fluoroetil)piperazin-1-il]-1,1'-bifenil-4-il}etil)-L-leucinamida;
- N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4-[4-(4-metilpiperazin-1-il)carbonil]fenil}etil)-L-leucinamida;
- 25 • N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-{[4-(2-hidroxi-2-metilpropil)piperazin-1-il]carbonil}fenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metil-1,3-tiazol-4-il)fenil]etil}-L-leucinamida;
- N²-{1-[4-(3-*terc*-butil-1,2,4-triazin-5-il)fenil]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
- 30 • N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-{2-[3-(metilsulfonil)fenil]-1,3-tiazol-4-il}fenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4-[2-(1H-pirazol-4-il)-1,3-tiazol-4-il]fenil}etil)-L-leucinamida;
- 35 • N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4'-[4-(metilsulfonil)piperazin-1-il]-

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- 1,1'-bifenil-4-il}etil)-L-leucinamida;
• N²-[1-(3-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
• N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-3-il]etil}-L-leucinamida;
5 • N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(3-piridin-4-ilfenil)etil]-L-leucinamida;
 • N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4'-piperazin-1-il-1,1'-bifenil-3-il)etil]-L-leucinamida;
 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-3-il]etil}-L-leucinamida;
10 • N-(cianometil)-1-[(2,2,2-trifluoro-1-feniletil)amino]ciclohexanocarboxamida;
 1-{[1-(4-bromofenil)-2,2,2-trifluoroetil]amino}-N-(cianometil)ciclohexanocarboxamida;
 • N-(cianometil)-1-{[2,2,2-trifluoro-1-(4'-piperazin-1-il-1,1'-bifenil-4-il)etil]amino}ciclohexanocarboxamida;
15 • N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-piperidin-4-ilfenil)etil]-L-leucinamida;
 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4-(4-piridin-2-ilpiperazin-1-il)fenil]etil}-L-leucinamida;
 • N²-[1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-3-ciclopropilalaninamida;
20 • N¹-(cianometil)-3-ciclopropil-N²-[2,2,2-trifluoro-1-(4-piridin-4-ilfenil)etil]alaninamida;
 • N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4'-piridin-4-il-1,1'-bifenil-4-il)etil]-L-leucinamida;
25 • N¹-(cianometil)-N²-[(1R)-2,2,2-trifluoro-1-(1,3-tiazol-2-il)etil]-L-leucinamida;
 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4'-metoxi-1,1'-bifenil-4-il)etil]-L-leucinamida;
 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-metoxifenil)etil]-L-leucinamida;
30 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4'-piridin-4-il-1,1'-bifenil-4-il)etil]-L-leucinamida;
 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-fenoxifenil)etil]-L-leucinamida;
 • N²-[(1S)-1-(4'-bromo-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
35 • N²-{(1S)-1-[4-(4-cloropiridin-3-il)fenil]-2,2,2-trifluoroetil}-N¹-(cianometil)- 121

- L-leucinamida;
 • N²-{(1S)-1-[4'-(acetilamino)-2'-metil-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
 • N²-[(1S)-1-(1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
 5 L-leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metoxipiridin-3-il)fenil]etil}-L-leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metoxipiridin-2-il)fenil]etil}-L-leucinamida;
 10 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4''-(metilsulfonyl)-1,1':4',1''-terfenil-4-il]etil}-L-leucinamida;
 • N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-3-(1-metilciclopropil)-L-alaninamida;
 • N¹-(cianometil)-3-(1-metilciclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-alaninamida;
 15 (metiltio)-1,1'-bifenil-4-il]etil}-L-alaninamida;
 • N¹-(cianometil)-3-(1-metilciclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-alaninamida;
 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4'-metil-1,1'-bifenil-4-il)etil]-L-leucinamida;
 20 • N²-[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(hidroximetil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 • N²-[1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-D-leucinamida;
 25 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-D-leucinamida;
 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4'-(morfolin-4-ilosulfonyl)-1,1'-bifenil-4-il]etil}-D-leucinamida;
 • N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4'-[(metilamino)sulfonyl]-1,1'-bifenil-4-il}etil)-D-leucinamida;
 30 • N¹-(cianometil)-N²-{(1R)-2,2,2-trifluoro-1-[4-(1-oxidopiridin-4-il)fenil]etil}-L-leucinamida;
 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4-(1-oxidopiridin-4-il)fenil]etil}-L-leucinamida;
 35 • N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4-[6-(1-hidroxi-1-metiletil)-1-

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- oxidopiridin-3-il]fenil}etil)-L-leucinamida;
- N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4-[6-(metilsulfonil)piridin-3-il]fenil}etil)-L-leucinamida;
- N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4-[2-(4-metilpiperazin-1-il)-1,3-tiazol-5-4-il]fenil}etil)-L-leucinamida;
- N²-[1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-L-leucinamida;
- N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-piperazin-1-ilfenil)etil]-L-leucinamida;
- 10 • N²-{1-[3'-(acetilamino)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-(2,2,2-trifluoro-1-[4-(4-propilpiperazin-1-il)fenil]etil)-L-leucinamida;
- N¹-(cianometil)-N²-(2,2,2-trifluoro-1-[4-(piperazin-1-ilcarbonil)fenil]etil)-L-leucinamida;
- 15 • N¹-(cianometil)-N²-[2,2,2-trifluoro-1-(4-{[4-(2-hidroxi)etil]piperazin-1-il}carbonil}fenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-{3-[3-(trifluorometil)fenil]-1,2,4-oxadiazol-5-il}fenil)etil]-L-leucinamida;
- 20 • Éster metílico del ácido 4-{1-[1-(cianometil-carbamoil)-3-metil-butilamino]-2,2,2-trifluoro-etil}-benzoico;
- N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4-[(E)-2-quinolin-2-iletetil]fenil}etil)-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(3-metil-1,2,4-oxadiazol-5-il)fenil]etil}-L-leucinamida;
- 25 • N²-((1S)-1-{4-[3-(5-bromopiridin-3-il)-1,2,4-oxadiazol-5-il]fenil}-2,2,2-trifluoroetil)-N¹-(cianometil)-L-leucinamida;
- N²-[(1S)-1-(4-benzoilfenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
- 30 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(tien-2-ilocarbonil)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1,3-tiazol-2-ilcarbonil)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-{(Z)-2-[4-(metilsulfonil)fenil]etenil}fenil)etil]-L-leucinamida;
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- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-{(E)-2-[4-(metilsulfonil)fenil]etenil} fenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-isobutirilfenil)etil]-L-leucinamida;
- 5 • N²-{(1S)-1-[4-(4-bromo-1,3-tiazol-2-il)fenil]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-1-(4-cianofenil)-2,2,2-trifluoroetil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-1-(4-etenilfenil)-2,2,2-trifluoroetil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(2'-fluoro-1,1'-bifenil-4-il)etil]-L-
- 10 leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1,3-tiazol-2-il)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 15 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4-(2-metilquinolin-7-il)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4-(1H-indol-5-il)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-{1-[4'-(dimetilamino)-1,1'-bifenil-4-il]-2,2,2-
- 20 trifluoroetil}-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-1-(4-{[(cianometil)amino]carbonil} fenil)-2,2,2-trifluoroetil]-L-leucinamida;
- N¹-(cianometil)-N²-[(1R)-1-(4-{[(cianometil)amino]carbonil} fenil)-2,2,2-trifluoroetil]-L-leucinamida;
- 25 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[3'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- ácido 4'-{1-[1-(Cianometil-carbamoil)-3-metil-butilamino]-2,2,2-trifluoroetil}-bifenil-4-carboxílico;
- Metoxi-metil-amida del ácido 4'-{1-[1-(cianometil-carbamoil)-3-metil-
- 30 butilamino]-2,2,2-trifluoro-etil}-bifenil-4-carboxílico;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-({[4-(metilsulfonil)bencil]tio} metil)fenil]etil}-L-leucinamida;
- N²-{(1S)-1-[4-(5-cloropiridin-2-il)fenil]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
- 35 • N²-{(1S)-1-[3'-(aminosulfonil)-4'-bromo-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-

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- N¹-(cianometil)-L-leucinamida;
• N²-{(1S)-1-[4'-bromo-3'-(metilsulfonil)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-
N¹-(cianometil)-L-leucinamida;
• N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4-[5-metil-6-
5 (metilsulfonil)piridin-3-il]fenil}etil)-L-leucinamida;
• N²-[(1S)-1-(4-{5-cloro-3-[4-(metilsulfonil)fenil]piridin-2-il}fenil)-2,2,2-
trifluoroetil]-N¹-(cianometil)-L-leucinamida;
• N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4-[(feniltio)metil]fenil}etil)-L-
leucinamida;
10 • N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4'-[(trifluorometil)sulfonil]-1,1'-
bifenil-4-il}etil)-L-leucinamida;
• N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-{[(4-
fluorobenzoil)amino]metil}fenil)etil]-L-leucinamida;
• N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(metilsulfonil)fenil]etil}-L-
15 leucinamida;
• N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-
bifenil-4-il]etil}-L-leucinamida;
• N¹-(cianometil)-N²-{(1S)-1-[4'-(etilsulfonil)-1,1'-bifenil-4-il]-2,2,2-
trifluoroetil}-L-leucinamida;
20 • N²-((1S)-1-{4-[(3-(2-cloro-6-fluorofenil)-5-metilisoxazol-4-
il]carbonil}amino)metil]fenil}-2,2,2-trifluoroetil)-N¹-(cianometil)-L-leucinamida;
• N²-((1S)-1-{4-[(9-cloro-3-metil-4-oxoisoxazolo[4,3-c]quinolin-5-(4H)-
il)metil]fenil}-2,2,2-trifluoroetil)-N¹-(cianometil)-L-leucinamida;
• N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-metoxi-3'-(metilsulfonil)-1,1'-
25 bifenil-4-il]etil}-L-leucinamida;
• N²-{(1S)-1-[4"-cloro-4'-(metilsulfonil)-1,1':2',1"-terfenil-4-il]-2,2,2-
trifluoroetil}-N¹-(cianometil)-L-leucinamida;
• N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[2'-metoxi-4'-(metilsulfonil)-1,1'-
bifenil-4-il]etil}-L-leucinamida;
30 • N²-{(1S)-1-[2'-cloro-4'-(metilsulfonil)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-
N¹-(cianometil)-L-leucinamida;
• N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4'-[(2-idroxi)etil]tio}-1,1'-bifenil-
4-il}etil)-L-leucinamida;
• N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[3'-fluoro-4'-(metilsulfonil)-1,1'-
35 bifenil-4-il]etil}-L-leucinamida;

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- N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4'-[(2-idroxi-1,1'-bifenil-4-il)etil]-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[3'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 5 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(2-[metoksi(metil)amino]-2-oxoetil)sulfonil]-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(cianometil)-N²-((1S)-2,2,2-trifluoro-1-{4'-[(2-idroxi-2-metilpropil)sulfonil]-1,1'-bifenil-4-il)etil}-L-leucinamida;
- N²-{(1S)-1-[4'-(aminosulfonil)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(1-
- 10 cianociclopropil)-L-leucinamida;
- N²-[(4-bromofenil) (2,4,6-trifluorofenil)metil]-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-[(4-piridin-4-ilfenil) (2,4,6-trifluorofenil)metil]-L-leucinamida;
- 15 • N¹-(cianometil)-N²-[[4-(4-fluorobencil)fenil](fenil)metil]-L-leucinamida;
- N¹-(cianometil)-N²-{fenilo[4-(piridin-3-ilmetil)fenil]metil}-L-leucinamida;
- N²-{(4-bromofenil)[4-(metilsulfonil)fenil]metil}-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-[[4-(metilsulfonil)fenil][4'-(metiltio)-1,1'-bifenil-4-il]etil]metil}-L-leucinamida;
- 20 • N¹-(cianometil)-N²-[[4'-(metilsulfonil)-1,1'-bifenil-4-il][4-(metilsulfonil)fenil]metil]-L-leucinamida;
- N¹-(cianometil)-N²-[2,2,2-tricloro-1-(4-glicoloilfenil)etil]-L-leucinamida;
- N¹-(cianometil)-N²-[2-fluoro-1-(fluorometil)-1-feniletil]-L-leucinamida;
- 25 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4-(pirrolidin-1-ilacetil)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4-(piperazin-1-ilcarbonil)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-(2,2,2-trifluoro-1-{4-[2-(4-metilpiperazin-1-il)-1,3-tiazol-4-il]fenil}etil)-L-leucinamida;
- 30 • N¹-(1-cianociclopropil)-3-(1-metilciclopropil)-N²-(2,2,2-trifluoro-1-{4-[1-(2-idroxi-1,1'-bifenil-4-il)etil]-L-leucinamida;
- N²-[[4-(4-terc-butilpiperazin-1-il)fenil](pentafluorofenil)metil]-N¹-(cianometil)-L-leucinamida;
- 35 • N-(cianometil)-1-{1-[4-(4-metilpiperazin-1-il)fenil]etil}piperidina-2- 2 |

- carboxamida;
- N²-[[4-(4-*terc*-butilpiperazin-1-il)fenil](piridin-2-il)metil]-N¹-(cianometil)-L-leucinamida;
 - N²-{[4-(4-*terc*-butilpiperazin-1-il)fenil][5-(trifluorometil)piridin-2-il]metil}-
 - 5 N¹-(cianometil)-L-leucinamida;
 - (4S)-N-(cianometil)-4-metil-1-[(1S)-1-(4-piperazin-1-ilfenil)etil]-L-prolinamida;
 - (4S)-N-(cianometil)-4-metil-1-[(1R)-1-(4-piperazin-1-ilfenil)etil]-L-prolinamida;
 - 10 • N-(cianometil)-1-[(1S)-1-(4-piperazin-1-ilfenil)etil]-L-prolinamida;
 - N-(cianometil)-1-[(1R)-1-(4-piperazin-1-ilfenil)etil]-L-prolinamida;
 - N-(cianometil)-4,4-difluoro-1-[(1S)-1-(4-piperazin-1-ilfenil)etil]-L-prolinamida;
 - N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-(4-metilfenil)etil]-L-
 - 15 leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1H-pirazol-3-il)fenil]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metil-1,3-oxazol-4-il)fenil]etil}-L-leucinamida;
 - 20 • N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(4-pirazin-2-ilfenil)etil]-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metilpiridin-4-il)fenil]etil}-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(4-metilpiridin-3-il)fenil]etil}-L-
 - 25 leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1H-pirazol-4-il)fenil]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-(4-piridin-4-ilfenil)etil]-L-leucinamida;
 - 30 • N²-[(1S)-1-(3'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-(3'-fluoro-4'-metil-1,1'-bifenil-4-il)etil]-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-{5-[4-(1-hidroxi-1-
 - 35 metiletil)fenil]piridin-2-il}etil]-L-leucinamida;

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- N¹-(cianometil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4'-(1-idroxi-1-metiletil)-1,1'-bifenil-4-il]propil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-[(1S)-2,2,3,3,3-pentafluoro-1-(4'-metil-1,1'-bifenil-4-il)propil]-L-leucinamida;
- 5 • N¹-(cianometil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4-(6-metoxipiridin-3-il)fenil]propil}-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,3,3,3-pentafluoro-1-(2'-fluoro-1,1'-bifenil-4-il)propil]-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-metoksi-3'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 10 • N²-{(1S)-1-[3'-(aminosulfonil)-4'-metoksi-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(1-cianociclopropil)-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metoxipiridin-3-il)fenil]etil}-L-leucinamida;
- 15 • N¹-(cianometil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4-(5-metilpiridin-2-il)fenil]propil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-{4-[5-(metilsulfonil)piridin-2-il]fenil}etil]-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(5-metilpiridin-2-il)fenil]etil}-L-leucinamida;
- 20 • N¹-(cianometil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 25 • N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[2'-metil-4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[5-(1H-pirazol-3-il)piridin-2-il]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-(5-quinolin-5-ilpiridin-2-il)etil]-L-leucinamida;
- 30 • N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-(5-quinolin-6-ilpiridin-2-il)etil]-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2-difluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 35 • N²-[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,3,3,3-pentafluoropropil]-N¹-(1-

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- cianociclopropil)-L-leucinamida;
- N²-[(1S)-1-(1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-L-leucinamida;
 - N²-{(1S)-1-[4'-(aminosulfonil)-1,1'-bifenil-4-il]-2,2,3,3,3-pentafluoropropil}-
- 5 N¹-(1-cianociclopropil)-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-1-[4-(1-etoxivinil)fenil]-2,2,2-trifluoroetil}-L-leucinamida;
 - N²-[(1S)-1-(4-acetilfenil)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-L-leucinamida;
- 10 • N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-(4-isopropilfenil)etil]-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-feniletil]-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1-hidroxi-1-metiletil)fenil]etil}-L-leucinamida;
- 15 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1-metilciclopropil)fenil]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-(2',4',6'-trimetil-1,1'-bifenil-4-il)etil]-L-leucinamida;
 - N²-[(1S)-1-(6-cloropiridin-3-il)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-L-
- 20 leucinamida;
- N²-{(1S)-1-[5-(4-acetilfenil)piridin-2-il]-2,2,2-trifluoroetil}-N¹-(1-cianociclopropil)-L-leucinamida;
 - N²-{(1S)-1-[6-(4-acetilfenil)piridin-3-il]-2,2,2-trifluoroetil}-N¹-(1-cianociclopropil)-L-leucinamida;
- 25 • N²-{(1S)-1-[5-(3-acetilfenil)piridin-2-il]-2,2,2-trifluoroetil}-N¹-(1-cianociclopropil)-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-((1S)-2,2,2-trifluoro-1-{5-[4-(1-hidroxi)etil]fenil]piridin-2-il}etil)-L-leucinamida;
 - N²-[(1S)-1-(1,1'-bifenil-4-il)-2,2,3,3,3-pentafluoropropil]-N¹-(cianometil)-L-
- 30 leucinamida;
- N²-[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,3,3,3-pentafluoropropil]-N¹-(cianometil)-L-leucinamida;
 - N²-[(1S)-1-(1,1'-bifenil-4-il)-2,2,3,3,3-pentafluoropropil]-N¹-(1-cianociclopropil)-L-leucinamida;
- 35 • N²-(1-bencil-2,2,2-trifluoroetil)-N¹-(1-cianociclopropil)-L-leucinamida;

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- XHX
- N²-[(1S)-1-(4-*terc*-butilfenil)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-L-leucinamida;
 - N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-4-metil-L-leucinamida;
 - 5 • N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-4-metil-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-((1S)-2,2,2-trifluoro-1-{4-[2-(1H-pirazol-4-il)-1,3-tiazol-4-il]fenil}etil)-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metil-1,3-tiazol-4-il)fenil]etil}-L-leucinamida;
 - 10 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metilpiridin-4-il)fenil]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metilpiridin-3-il)fenil]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metilpiridin-3-il)fenil]etil}-L-leucinamida;
 - 15 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metilpiridin-2-il)fenil]etil}-L-leucinamida;
 - N²-[(1S)-1-(3'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-4-fluoro-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-(1H-pirazol-3-il)fenil]etil}-L-leucinamida;
 - 20 • N¹-[(1S)-1-cianoetil]-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-[(1S)-1-ciano-3-(metiltio)propil]-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - 25 • N¹-[(1S)-1-ciano-3-(metilsulfonil)propil]-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N²-[(1S)-1-(4-bromofenil)-2,2,3,3,3-pentafluoropropil]-N¹-(1-cianociclopropil)-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4-(6-metoxipiridin-2-il)fenil]propil}-L-leucinamida;
 - 30 • N²-[(1S)-1-(5-bromopiridin-2-il)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-{5-[4-(metilsulfonil)fenil]piridin-2-il}etil)-L-leucinamida;
 - 35 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(1-idroxi-1-metiletil)-

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- 1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(cianometil)-N²-[(1S)-2,2,2-trifluoro-1-(6'-metil-3,3'-bipiridin-6-il)etil]-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metoxipiridin-2-il)fenil]etil}-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-oxo-1,6-dihidropiridin-2-il)fenil]etil}-L-leucinamida;
 - (4S)-N¹-(cianometil)-5,5,5-trifluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - (4S)-N¹-(1-cianociclopropil)-5,5,5-trifluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - (4S)-N¹-(cianometil)-5,5,5-trifluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - (4S)-N¹-(1-cianociclopropil)-5,5,5-trifluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - (4S)-N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-5,5,5-trifluoro-L-leucinamida;
 - (4S)-N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-5,5,5-trifluoro-L-leucinamida;
 - N²-{(1S)-1-[4-(6-aminopiridin-3-il)fenil]-2,2,2-trifluoroetil}-N¹-(cianometil)-L-leucinamida;
 - N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-4-fluoro-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4-(6-metilpiridin-3-il)fenil]propil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4-(6-metilpiridin-3-il)fenil]propil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metilpiridin-3-il)fenil]etil}-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(1-idroxi-etil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(2,2,2-trifluoro-1-idroxi-etil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]propil}-L-leucinamida;

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- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4'-(1-hidroxi-1-metiletil)-1,1'-bifenil-4-il]propil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]propil}-L-leucinamida;
- 5 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4-(6-metoxipiridin-2-il)fenil]propil}-L-leucinamida;
- (4R)-N¹-(cianometil)-5,5,5-trifluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- (4R)-N¹-(1-cianociclopropil)-5,5,5-trifluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 10 • N¹-(cianometil)-N²-[(1S)-2,2,3,3,3-pentafluoro-1-(4'-metil-1,1'-bifenil-4-il)propil]-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4-(1,3-tiazol-2-il)fenil]propil}-L-leucinamida;
- 15 • N¹-(1-cianociclopropil)-N²-[(1S)-1-(4-etinilfenil)-2,2,3,3,3-pentafluoropropil]-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-1-[4-(ciclopropiletinil)fenil]-2,2,2-trifluoroetil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-1-[4-(ciclopropiletinil)fenil]-2,2,2-trifluoroetil}-L-leucinamida;
- 20 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(5-metil-1,3-tiazol-2-il)fenil]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1,3-tiazol-2-il)fenil]etil}-L-leucinamida;
- 25 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(5-metil-1,3-tiazol-2-il)fenil]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-1-[4-(ciclopropiletinil)fenil]-2,2,3,3,3-pentafluoropropil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(4-metil-1,3-tiazol-2-il)fenil]etil}-L-leucinamida;
- 30 • N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(4-metil-1,3-tiazol-2-il)fenil]etil}-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-1-[4-(4,5-dimetil-1,3-tiazol-2-il)fenil]-2,2,2-trifluoroetil}-L-leucinamida;
- 35 • N¹-(1-cianociclopropil)-N²-{(1S)-1-[4'-(etilsulfonyl)-1,1'-bifenil-4-il]-2,2,2-

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- trifluoroetil}-4-fluoro-L-leucinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-[(1S)-2,2,2-trifluoro-1-(4-piridin-3-ilfenil)etil]-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-metoxi-3'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - 5 (metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-alaninamida;
 - N²-{(1S)-1-[4'-(aminosulfonyl)-1,1'-bifenil-4-il]-2,2,3,3,3-pentafluoropropil}-N¹-(cianometil)-L-leucinamida;
 - N¹-(cianometil)-N²-((1S)-2,2,3,3,3-pentafluoro-1-{4'-[(2-hidroxi-2-metilpropil)sulfonyl]-1,1'-bifenil-4-il}propil)-L-leucinamida;
 - 10 metilpropil)sulfonyl]-1,1'-bifenil-4-il}propil)-L-leucinamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-alaninamida;
 - N¹-(cianometil)-N²-{(1S)-2,2,3,3,3-pentafluoro-1-[4'-(isopropilsulfonyl)-1,1'-bifenil-4-il]propil}-L-leucinamida;
 - 15 • N¹-(1-ciano-1-metiletil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-{4'-[(2-hidroxi-2-metilpropil)sulfonyl]-1,1'-bifenil-4-il}etil)-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[2'-metil-4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - 20 1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-1-[4'-(etilsulfonyl)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-L-leucinamida;
 - N²-{(1S)-1-[4'-(aminosulfonyl)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(1-cianociclopropil)-4-fluoro-L-leucinamida;
 - 25 • N¹-(cianometil)-N²-{(S)-[4'-(metilsulfonyl)-1,1'-bifenil-4-il][4-(trifluorometoxi)fenil]metil}-L-leucinamida;
 - N¹-(cianometil)-N²-[(S)-[4'-(metilsulfonyl)-1,1'-bifenil-4-il](tien-2-il)metil]-L-leucinamida;
 - metanosulfonato de N¹-(cianometil)-N²-[(S)-(4'-piperazin-4-io-1-il-1,1'-bifenil-4-il) (tien-2-il)metil]-L-leucinamida;
 - 30 4-il) (tien-2-il)metil]-L-leucinamida;
 - N¹-(cianometil)-N²-{(S)-(4-fluorofenil)[4'-(metilsulfonyl)-1,1'-bifenil-4-il]metil}-L-leucinamida;
 - N¹-(cianometil)-N²-{(S)-[4'-(metilsulfonyl)-1,1'-bifenil-4-il][4-(trifluorometil)fenil]metil}-L-leucinamida;
 - 35 • N²-{(S)-(4-clorofenil)[4'-(metilsulfonyl)-1,1'-bifenil-4-il]metil}-N¹-

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- (cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
 - N²-[(S)-(4-bromofenil) (tien-2-il)metil]-N¹-(cianometil)-L-leucinamida;
- 5 • N¹-(cianometil)-N²-[(S)-[4-(4,4,5,5-tetrametil-1,3,2-dioxaborolan-2-il)fenil](tien-2-il)metil]-L-leucinamida;
- N²-{(R)-(4-bromofenil)[4-(trifluorometoxi)fenil]metil}-N¹-(cianometil)-L-leucinamida;
 - N¹-(cianometil)-N²-{(S)-[4-(4,4,5,5-tetrametil-1,3,2-dioxaborolan-2-il)fenil][4-(trifluorometoxi)fenil]metil}-L-leucinamida;
- 10 • N²-[(S)-(4-bromofenil) (2-furil)metil]-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-{(S)-2-furil[4'-(metilsulfonil)-1,1'-bifenil-4-il]metil}-L-leucinamida;
 - N²-[1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-L-norvalinamida;
- 15 • N²-{(R)-(4-bromofenil)[4-(trifluorometil)fenil]metil}-N¹-(cianometil)-L-leucinamida;
- N¹-(cianometil)-N²-{1-[4'-(4-ciclopropilpiperazin-1-il)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-L-norvalinamida;
 - N²-[(R)-(4-bromofenil) (4-clorofenil)metil]-N¹-(cianometil)-L-leucinamida;
- 20 • N¹-(cianometil)-N²-{2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N²-[(S)-(4-bromofenil) (3-metiltien-2-il)metil]-N¹-(cianometil)-L-leucinamida;
 - N²-[(S)-(4-bromofenil) (tien-3-il)metil]-N¹-(cianometil)-L-leucinamida;
 - N¹-(cianometil)-N²-{(S)-(2,4-difluorofenil)[4'-(metilsulfonil)-1,1'-bifenil-4-il]metil}-L-leucinamida;
- 25 • N¹-(cianometil)-N²-[(S)-[4'-(metilsulfonil)-1,1'-bifenil-4-il](tien-3-il)metil]-L-leucinamida;
- N¹-(cianometil)-N²-[(S)-[4'-(metilsulfonil)-1,1'-bifenil-4-il](3-metiltien-2-il)metil]-L-leucinamida;
- 30 • N¹-(cianometil)-N²-[(S)-[4'-(4-ciclopropilpiperazin-1-il)-1,1'-bifenil-4-il](3-metiltien-2-il)metil]-L-leucinamida;
- N¹-(cianometil)-N²-[(S)-[4'-(4-ciclopropilpiperazin-1-il)-1,1'-bifenil-4-il](tien-3-il)metil]-L-leucinamida;
 - N²-[1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-5,5,5-trifluoro-L-
- 35 norvalinamida;

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- N¹-(cianometil)-5,5,5-trifluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N²-[(S)-(4-bromofenil) (3-metiltien-2-il)metil]-N¹-(1-cianociclopropil)-L-leucinamida;
- 5 • N¹-(1-cianociclopropil)-N²-[(S)-[4'-(metilsulfonyl)-1,1'-bifenil-4-il](3-metiltien-2-il)metil]-L-leucinamida;
- N¹-(cianometil)-N²-{(S)-3-furil[4'-(metilsulfonyl)-1,1'-bifenil-4-il]metil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- 10 • N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-L-norvalinamida N²-[(S)-(4-bromofenil) (4-bromotien-2-il)metil]-N¹-(cianometil)-L-leucinamida;
- N²-[(S)-(4-bromofenil) (tien-3-il)metil]-N¹-(1-cianociclopropil)-L-leucinamida;
- 15 • N¹-(cianometil)-N²-[(S)-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]{4-[4-(metilsulfonyl)fenil]tien-2-il}metil]-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-[(S)-[4'-(metilsulfonyl)-1,1'-bifenil-4-il](tien-3-il)metil]-L-leucinamida;
- 20 • N²-{(1S)-1-[4'-(aminosulfonyl)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-N¹-(1-cianociclopropil)-L-norvalinamida;
- N²-[(S)-(4-bromofenil) (4-bromotien-2-il)metil]-N¹-(1-cianociclopropil)-L-leucinamida;
- N²-[(S)-[4'-(aminosulfonyl)-1,1'-bifenil-4-il](tien-3-il)metil]-N¹-(1-cianociclopropil)-L-leucinamida;
- 25 • N²-[(S)-[4'-(aminosulfonyl)-1,1'-bifenil-4-il](tien-3-il)metil]-N¹-(cianometil)-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-metoxi-3'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- 30 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metilpiridin-4-il)fenil]etil}-L-norvalinamida;
- N¹-(1-cianociclopropil)-5,5,5-trifluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(1H-pirazol-3-il)fenil]etil}-L-norvalinamida;
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- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(1-hidroxi-1-metiletil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(5-metilpiridin-2-il)fenil]etil}-L-norvalinamida;
- 5 • Cianometil-amida del ácido 2-[[4-bromo-fenil]-piridin-4-il-metil]-amino}-pentanoico;
- Cianometil-amida del ácido 2-[[4-bromo-fenil]-tiazol-2-il-metil]-amino}-pentanoico;
- (1-cianociclopropil)-amida del ácido (2S)-2-[(S)-1-(4'-acetilbifenil-4-il)-2,2,2-trifluoroetilamino]-pentanoico;
- 10 • (1-cianociclopropil)-amida del ácido (2S)-2-[(S)-1-(2',4'-difluorobifenil-4-il)-2,2,2-trifluoroetilamino]-pentanoico;
- (1-cianociclopropil)-amida del ácido (2S)-2-[(S)-1-(3',4'-difluorobifenil-4-il)-2,2,2-trifluoroetilamino]-pentanoico;
- 15 • (1-ciano-ciclopropil)-amida del ácido (2S)-2-[(S)-1-(3'-cloro-4'-fluorobifenil-4-il)-2,2,2-trifluoroetilamino]-pentanoico;
- (1-Ciano-ciclopropil)-amida del ácido (2S)-2-[(S)-2,2,2-trifluoro-1-(4'-metanosulfonilamino-bifenil-4-il)-etilamino]-pentanoico;
- Cianometil-amida del ácido (2S)-2-[(S)-[(4-bromo-fenil)-tiazol-2-il-metil]-amino}-4-metilpentanoico;
- 20 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-cloro-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-cloro-3'-metil-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- 25 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-cloro-2'-metil-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- (1-Ciano-ciclopropil)-amida del ácido (2S)-2-[(S)-2,2,2-trifluoro-1-[4-(1H-indol-5-il)-fenil]-etilamino]-pentanoico;
- (1-Ciano-ciclopropil)-amida del ácido (2S)-2-[(S)-2,2,2-trifluoro-1-(3'-metanosulfonilamino-bifenil-4-il)-etilamino]-pentanoico;
- 30 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-fluoro-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-fluoro-3'-metil-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- 35 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[3'-fluoro-4'-metil-1,1'-

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- bifenil-4-il]etil}-L-norvalinamida;
• N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-trifluorometoxi-1,1'-
bifenil-4-il]etil}-L-norvalinamida;
• (1-Cianociclopropil)-amida del ácido (2S)-2-[(S)-2,2,2-trifluoro-1-(4'-
5 metilbifenil-4-il)-etilamino]-pentanoico;
• (1-Cianociclopropil)-amida del ácido (2S)-2-[(S)-1-(4'-cianobifenil-4-il)-
2,2,2-trifluoroetilamino]-pentanoico;
• N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-metoxi-1,1'-bifenil-4-
il]etil}-L-norvalinamida;
10 • N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4-(benzo[1,3]dioxol-5-
il)fenil]etil}-L-norvalinamida;
• N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metoxicarbonil)-1,1'-
bifenil-4-il]etil}-L-norvalinamida;
• (1-Cianociclopropil)-amida del ácido (2S)-2-[(S)-[(4-bromofenil)-tiazol-2-il-
15 metil]-amino]-4-metilpentanoico;
• Cianometil-amida del ácido (2S)-2-[(S)-[(4'-metanosulfonil-bifenil-4-il)-
tiazol-2-il-metil]-amino]-4-metil-pentanoico;
• (1-Ciano-ciclopropil)-amida del ácido (2S)-2-[(S)-2,2,2-trifluoro-1-(4'-
trifluorometil-bifenil-4-il)-etilamino]-pentanoico;
20 • (1-Ciano-ciclopropil)-amida del ácido (2S)-2-[(S)-2,2,2-trifluoro-1-(2'-
trifluorometil-bifenil-4-il)-etilamino]-pentanoico;
• (1-Cianociclopropil)-amida del ácido (2S)-2-[(S)-[(2',4'-difluorobifenil-4-il)-
tiazol-2-il-metil]-amino]-4-metilpentanoico;
• (1-Cianociclopropil)-amida del ácido (2S)-2-[(S)-[(4'-metanosulfonilbifenil-4-
25 il)-tiazol-2-il-metil]-amino]-4-metilpentanoico;
• N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-(1-oxido-2,3-
dihidro-1-benzotien-5-il)fenil]etil}-L-leucinamida;
• N¹-(1-cianociclopropil)-N²-{(1S)-1-[4-(1,1-dioxido-2,3-dihidro-1-benzotien-5-
il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
30 • N¹-(1-cianociclopropil)-N²-{(1S)-1-[4-(1,1-dioxido-2,3-dihidro-1,2-
benzisotiazol-5-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
• N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfinil)-
1,1'-bifenil-4-il]etil}-L-leucinamida;
• N¹-(1-cianociclopropil)-N²-{(1S)-1-[4-(1,1-dioxido-3-oxo-2,3-dihidro-1,2-
35 benzisotiazol-5-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;

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- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[2'-(1-idroxi-1-metiletil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-4,4-difluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
 - 5 • N¹-(cianometil)-4,4-difluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
 - N¹-(cianometil)-4-fluoro-N²-{(1S)-2,2,2-tricloro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-tricloro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - 10 • N¹-(1-cianociclopropil)-4,4-difluoro-N²-{(1S)-2,2,2-tricloro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
 - N¹-(cianometil)-4,4-difluoro-N²-{(1S)-2,2,2-tricloro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
 - 15 • (2S)-N-(cianometil)-4,4-difluoro-2-((1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil)amino)butanamida;
 - (2S)-N-(1-cianociclopropil)-4,4-difluoro-2-((1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil)amino)butanamida;
 - (2S)-N-(1-cianociclopropil)-4,4-difluoro-2-((1S)-2,2,2-tricloro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil)amino)butanamida;
 - 20 • (2S)-N-(cianometil)-4,4-difluoro-2-((1S)-2,2,2-tricloro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil)amino)butanamida;
 - (2S)-4,4-dicloro-N-(cianometil)-2-((1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil)amino)butanamida;
 - 25 • (2S)-4,4-dicloro-N-(1-cianociclopropil)-2-((1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil)amino)butanamida;
 - (2S)-4,4-dicloro-N-(1-cianociclopropil)-2-((1S)-2,2,2-tricloro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil)amino)butanamida;
 - (2S)-4,4-dicloro-N-(cianometil)-2-((1S)-2,2,2-tricloro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil)amino)butanamida;
 - 30 • N²-[(1S)-1-(1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-4-fluoro-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[3'-(1-idroxi-1,1'-bifenil-4-il]etil)-L-leucinamida;
 - 35 • N¹-(1-cianociclopropil)-4-fluoro-N²-[(1S)-2,2,2-trifluoro-1-(4'-metil-1,1'-

- bifenil-4-il)etil]-L-leucinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[3'-(1-hidroxi-1-metiletil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N²-[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-4-fluoro-L-leucinamida;
 - N²-[(1S)-1-(2'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-4-fluoro-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(1-hidroxi-1,1'-bifenil-4-il)etil]-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[2'-(1-hidroxi-1,1'-bifenil-4-il)etil]-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(1-hidroxi-1-metiletil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[2'-(1-hidroxi-1-metiletil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-{5-[4-(metilsulfonyl)fenil]piridin-2-il}etil)-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-{6-[4-(metilsulfonyl)fenil]piridin-3-il}etil)-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-[(1S)-2,2,2-trifluoro-1-(4-{6-[(metilsulfonyl)amino]piridin-3-il}fenil)etil]-L-leucinamida;
 - N¹-(cianometil)-4-fluoro-N²-[(1S)-2,2,2-trifluoro-1-(4-{6-[(metilsulfonyl)amino]piridin-3-il}fenil)etil]-L-leucinamida;
 - N¹-(1-cianobutil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-ciano-2-ciclopropiletil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-ciano-2-piridin-3-iletil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-ciano-3-hidroxi-3-metilbutil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-(1-oxo-1,3-dihidro-2-benzofuran-5-il)fenil]etil}-L-leucinamida;
 - N¹-(1-cianociclopropil)-N²-{(1S)-1-[4-(3,3-dimetil-1-oxo-1,3-dihidro-2-benzofuran-5-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;

- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(3,3-dietil-1-oxo-1,3-dihidro-2-benzofuran-5-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4-(3-oxo-1,3-dihidro-2-benzofuran-5-il)fenil]etil}-L-leucinamida;
- 5 N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(1,1-dimetil-3-oxo-1,3-dihidro-2-benzofuran-5-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(1,1-dietil-3-oxo-1,3-dihidro-2-benzofuran-5-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- 10 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(3-oxo-1,3-dihidro-2-benzofuran-5-il)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4'-(1,1-dimetil-3-oxo-1,3-dihidro-2-benzofuran-5-il)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4'-(1,1-dietil-3-oxo-1,3-dihidro-2-benzofuran-5-il)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- 15 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(1-oxo-1,3-dihidro-2-benzofuran-5-il)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4'-(3,3-dimetil-1-oxo-1,3-dihidro-2-benzofuran-5-il)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4'-(3,3-dietil-1-oxo-1,3-dihidro-2-benzofuran-5-il)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- 20 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4-(5-oxo-2,5-dihidrofuran-3-il)fenil]etil}-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(2,2-dimetil-5-oxo-2,5-dihidrofuran-3-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- 25 N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(2,2-dietil-5-oxo-2,5-dihidrofuran-3-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4-(2-oxo-2,5-dihidrofuran-3-il)fenil]etil}-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(5,5-dimetil-2-oxo-2,5-dihidrofuran-3-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- 30 N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(5,5-dietil-2-oxo-2,5-dihidrofuran-3-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4-(5-oxo-4-oxaspiro[2.4]hept-6-en-7-il)fenil]etil}-L-leucinamida;
- 35 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4-(6-oxo-5-

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- oxaspiro[3.4]oct-7-en-8-il)fenil]etil}-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4-(5-oxo-4-oxaspiro[2.4]hept-6-en-6-il)fenil]etil}-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4-(6-oxo-5-oxaspiro[3.4]oct-7-en-7-il)fenil]etil}-L-leucinamida;
5 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4-quinolin-6-ilfenil)etil]-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfinil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
10 N^2 -[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]- N^1 -(1-cianociclopropil)-L-leucinamida;
 N^1 -(1-cianociclopropil)- N^2 -[(1S)-2,2,2-trifluoro-1-(4-quinolin-6-ilfenil)etil]-L-leucinamida;
 N^2 -[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]- N^1 -(1-cianociclopropil)-4,4-difluoro-L-norvalinamida;
15 N^1 -(1-cianociclopropil)-4,4-difluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
 N^1 -(1-cianociclopropil)-4,4-difluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
20 N^1 -[(1S)-1-ciano-3-(metilsulfonyl)propil]- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4-quinolin-6-ilfenil)etil]-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfinil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
25 N^1 -(1-cianociclopropil)- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(1-idroxietyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 N^2 -[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]- N^1 -(1-cianociclopropil)-L-leucinamida;
30 N^1 -(1-cianociclopropil)- N^2 -[(1S)-2,2,2-trifluoro-1-(4-quinolin-6-ilfenil)etil]-L-leucinamida;
 N^2 -[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]- N^1 -(1-cianociclopropil)-4,4-difluoro-L-norvalinamida;
 N^1 -(1-cianociclopropil)-4,4-difluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
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- N¹-(1-cianociclopropil)-4,4-difluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-[5-metil-6-(metilsulfonil)piridin-3-il]fenil]etil}-L-leucinamida;
- 5 N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-[6-(1-hidroxi-1-metiletil)-5-metilpiridin-3-il]fenil]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(fluoro-1,1'-bifenil-4-il)etil]-L-leucinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[3'-metil-4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 10 N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metilpiridin-3-il)fenil]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 15 N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(1-hidroxi-1-metiletil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-metilquinolin-7-il)fenil]etil}-L-leucinamida;
- N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-4,4-difluoro-L-
- 20 norvalinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-[(1S)-1-hidroxi-etil]-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-[(1R)-1-hidroxi-etil]-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 25 N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(trifluoroacetil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[5-(2-naftil)piridin-2-il]etil}-L-leucinamida;
- N¹-(cianometil)-4,4-difluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- 30 N¹-(cianometil)-4,4-difluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-(5-metil-1,3-tiazol-2-il)fenil]etil}-L-leucinamida;
- 35 N¹-(1-cianociclopropil)-N²-{(1S)-1-[4-(1,1-dioxido-3-oxo-2,3-dihidro-1,2-

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- benzisotiazol-5-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
 N^2 -[(4-bromofenil) (fenil)metil]- N^1 -(1-cianociclopropil)-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4'-metil-1,1'-bifenil-4-il)etil]-L-leucinamida;
5 N^2 -[(1S)-1-(1,1'-bifenil-4-il)-2,2,2-trifluoroetil]- N^1 -(1-cianociclopropil)-4-fluoro-L-leucinamida;
 N^2 -{(1S)-1-[4-(5-cloropiridin-2-il)fenil]-2,2,2-trifluoroetil}- N^1 -(1-cianociclopropil)-4-fluoro-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4-piridin-4-ilfenil)etil]-L-leucinamida;
10 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-{4'-[(metilsulfonyl)amino]-1,1'-bifenil-4-il}etil]-L-leucinamida;
 N^2 -[(1S)-1-(4-bromofenil)-2,2-difluoroetil]- N^1 -(1-cianociclopropil)-L-leucinamida;
15 N^1 -(1-cianociclopropil)- N^2 -{(1S)-2,2-difluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4-pirimidin-5-ilfenil)etil]-L-leucinamida;
 N^2 -[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]- N^1 -(1-cianociclopropil)-4-fluoro-L-leucinamida;
20 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[3'-(1-hidroxietil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 N^2 -[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2-difluoroetil]- N^1 -(1-cianociclopropil)-L-leucinamida;
25 N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(3,5-dimetilisoxazol-4-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
 N^1 -[(1S)-1-ciano-3-(metilsulfonyl)propil]- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonyl)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4-quinolin-6-ilfenil)etil]-L-leucinamida;
30 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfinil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
 N^1 -(1-cianociclopropil)- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(1-hidroxietil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
35 N^2 -[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]- N^1 -(1-

- cianociclopropil)-L-leucinamida;
N¹-(1-cianociclopropil)-N²-[(1S)-2,2,2-trifluoro-1-(4-quinolin-6-ilfenil)etil]-L-leucinamida;
N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(1-cianociclopropil)-4,4-
5 difluoro-L-norvalinamida;
N¹-(1-cianociclopropil)-4,4-difluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
N¹-(1-cianociclopropil)-4,4-difluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
10 N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-[5-metil-6-(metilsulfonil)piridin-3-il]fenil]etil}-L-leucinamida;
N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-[6-(1-idroxi-1-metiletil)-5-metilpiridin-3-il]fenil]etil}-L-leucinamida;
N¹-(1-cianociclopropil)-4-fluoro-N²-[(1S)-2,2,2-trifluoro-1-(4'-fluoro-1,1'-
15 bifenil-4-il)etil]-L-leucinamida;
N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[3'-metil-4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-(6-metilpiridin-3-il)fenil]etil}-L-leucinamida;
20 N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-leucinamida;
N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-(1-idroxi-1-metiletil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4-(2-
25 metilquinolin-7-il)fenil]etil}-L-leucinamida;
N²-[(1S)-1-(4-bromofenil)-2,2,2-trifluoroetil]-N¹-(cianometil)-4,4-difluoro-L-norvalinamida;
N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-[(1S)-1-idroxi-1,1'-bifenil-4-il]etil]-L-leucinamida;
30 N¹-(1-cianociclopropil)-4-fluoro-N²-{(1S)-2,2,2-trifluoro-1-[4'-[(1R)-1-idroxi-1,1'-bifenil-4-il]etil]-L-leucinamida;
N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[4'-(trifluoroacetil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
N¹-(1-cianociclopropil)-N²-{(1S)-2,2,2-trifluoro-1-[5-(2-naftil)piridin-2-
35 il]etil}-L-leucinamida;

- N^1 -(cianometil)-4,4-difluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- N^1 -(cianometil)-4,4-difluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-norvalinamida;
- 5 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[4-(5-metil-1,3-tiazol-2-il)fenil]etil}-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(1,1-dioxido-3-oxo-2,3-dihidro-1,2-benzisotiazol-5-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- N^2 -[(4-bromofenil)(fenil)metil]- N^1 -(1-cianociclopropil)-L-leucinamida;
- 10 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4'-metil-1,1'-bifenil-4-il)etil]-L-leucinamida;
- N^2 -[(1S)-1-(1,1'-bifenil-4-il)-2,2,2-trifluoroetil]- N^1 -(1-cianociclopropil)-4-fluoro-L-leucinamida;
- N^2 -{(1S)-1-[4-(5-cloropiridin-2-il)fenil]-2,2,2-trifluoroetil}- N^1 -(1-cianociclopropil)-4-fluoro-L-leucinamida;
- 15 N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4-piridin-4-ilfenil)etil]-L-leucinamida;
- N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-{4'-[(metilsulfonil)amino]-1,1'-bifenil-4-il}etil]-L-leucinamida;
- 20 N^2 -[(1S)-1-(4-bromofenil)-2,2-difluoroetil]- N^1 -(1-cianociclopropil)-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-2,2-difluoro-1-[4'-(metiltio)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- N^1 -(1-cianociclopropil)-4-fluoro- N^2 -[(1S)-2,2,2-trifluoro-1-(4-pirimidin-5-ilfenil)etil]-L-leucinamida;
- 25 N^2 -[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2,2-trifluoroetil]- N^1 -(1-cianociclopropil)-4-fluoro-L-leucinamida;
- N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1S)-2,2,2-trifluoro-1-[3'-(1-hidroxietil)-1,1'-bifenil-4-il]etil}-L-leucinamida;
- 30 N^2 -[(1S)-1-(4'-acetil-1,1'-bifenil-4-il)-2,2-difluoroetil]- N^1 -(1-cianociclopropil)-L-leucinamida;
- N^1 -(1-cianociclopropil)- N^2 -{(1S)-1-[4-(3,5-dimetilisoxazol-4-il)fenil]-2,2,2-trifluoroetil}-4-fluoro-L-leucinamida;
- y las sales, estereoisómeros y derivados de N-óxido farmacéuticamente
- 35 aceptables de los mismos.

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10. N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1*S*)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida o una sal farmacéuticamente aceptable de la misma.
- 5
11. N^1 -(1-cianociclopropil)-4-fluoro- N^2 -{(1*S*)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida o una sal farmacéuticamente aceptable de la misma.
- 10
12. N^1 (cianometil)- N^2 {(1*S*)-2,2,2-trifluoro-1-[4'-(metilsulfonil)-1,1'-bifenil-4-il]etil}-L-leucinamida o una sal farmacéuticamente aceptable de la misma.
13. N^2 {(1*S*)-1-[4'-(aminosulfonil)-1,1'-bifenil-4-il]-2,2,2-trifluoroetil}- N^1 (cianometil)-L-leucinamida o una sal farmacéuticamente aceptable de la misma.
- 15
14. Una composición farmacéutica que comprende un compuesto de acuerdo con la reivindicación 1, en una cantidad terapéutica eficaz, y un vehículo farmacéuticamente aceptable.
- 20
15. Una composición farmacéutica que comprende un compuesto de la Reivindicación 1 y otro agente seleccionado entre: alendronato, cimadronato, clodronato, etidronato, ibandronato, incadronato, minodronato, neridronato, olpadronato, pamidronato, piridronato, risedronato, tiludronato, zolendronato; estrógeno, progestrógeno, estradiol, droloxifeno, raloxifeno, lasofoxifeno, TSE-424,
- 25
- tamoxifeno, idoxifeno, LY353381, LY117081, toremifeno, fulvestrant, 4-[7-(2,2-dimetil-1-oxopropoxi-4-metil-2-[4-[2-(1-piperidinil)etoxi]fenil]-2H-1-benzopiran-3-il]-fenil-2,2-dimetilpropanoato, 4,4'-dihidroxibenzofenona-2,4-dinitrofenil-hidrazona, SH646; finasterida y otros inhibidores de 5 α -reductasa, nilutamida, flutamida, bicalutamida, liarozol, acetato de abiraterona; lovastatina, simvastatina,
- 30
- pravastatina, fluvastatina, atorvastatina, cerivastatina; hormona paratiroide (PTH); y las sales y mezclas farmacéuticamente aceptables de los mismos.



Trifluoroethylamines as amide isosteres in inhibitors of cathepsin K

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Received 13 June 2005; revised 15 July 2005; accepted 25 July 2005

Available online 9 September 2005

Abstract—The P2-P3 amide of dipeptide cathepsin K inhibitors can be replaced by the metabolically stable trifluoroethylamine group. The non-basic nature of the nitrogen allows the important hydrogen bond to Gly66 to be made. The resulting compounds are 10- to 20-fold more potent than the corresponding amide derivatives. Compound **8** is a 5 pM inhibitor of human cathepsin K with >10,000-fold selectivity over other cathepsins.

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Cathepsin K (Cat K) is a lysosomal cysteine protease thought to be the principal enzyme responsible for degradation of type I collagen in osteoclastic bone resorption. Thus, an inhibitor of Cat K may be effective as an anti-resorptive for treatment of osteoporosis. A number of Cat K inhibitors have been previously reported, many of which contain multiple amide bonds.

Replacement of amide bonds with appropriate isosteres is a classic approach in medicinal chemistry. Many amide replacements are known which retain the geometry of the amide bond or maintain the hydrogen bond-accepting properties of the amide.¹ However, there are a few functional groups, which are capable of preserving the hydrogen bond-donating properties of the amide. Sulfonamides, anilines, secondary alcohols, hydrazines, and certain heterocycles constitute this list. The problem of identifying an NH amide replacement can be recast as how to minimize the basicity of an NH donor so that a NH_2^+ moiety is not formed in the biological milieu. Such charged groups are poorly tolerated deep in the active site of a protein where binding interactions cannot compensate for the energetic cost of desolvation. In this paper, we describe that a trifluoromethyl group can

replace the carbonyl of an amide and generate a metabolically stable, non-basic amine that maintains the excellent hydrogen bond of an amide.

Replacement of this functional group has been previously explored by Zanda and coworkers² who have used it to replace both a glycine amide bond and a malonamide of a partially modified retropeptide.³ These authors succinctly note that the trifluoroethylamino group features 'low NH basicity, a $\text{CH}(\text{CF}_3)\text{—NH—CH}$ backbone angle close to 120° , [and] a C—CF_3 bond substantially isopolar with the C=O '. A report on the use of a $\text{CH}(\text{CN})\text{—NH}$ group to replace an amide bond in CCK-B has also been presented.⁴

Much of the published literature on Cat K inhibitors describe peptide-based molecules. Dipeptide nitrile inhibitors have been reported with good pharmacokinetics in preclinical species and anti-resorptive activity in animal models of osteoporosis.⁵ Nonetheless, we felt that the overall properties of these inhibitors could be improved by decreasing their peptidic nature.

X-ray crystallography of the irreversible dipeptide inhibitor **1** bound to Cat K shows that the P1-P2 amide forms hydrogen bonds to the protein with both its NH and carbonyl components (Fig. 1). In contrast, the P2-P3 amide bond forms only one hydrogen bond to Gly66

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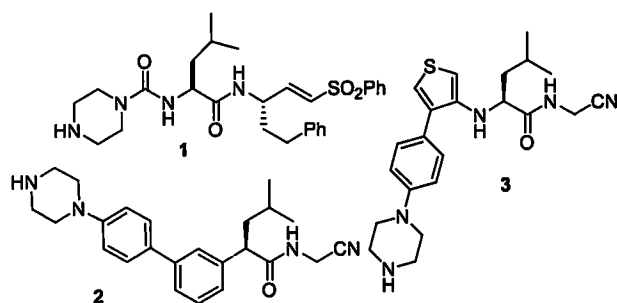


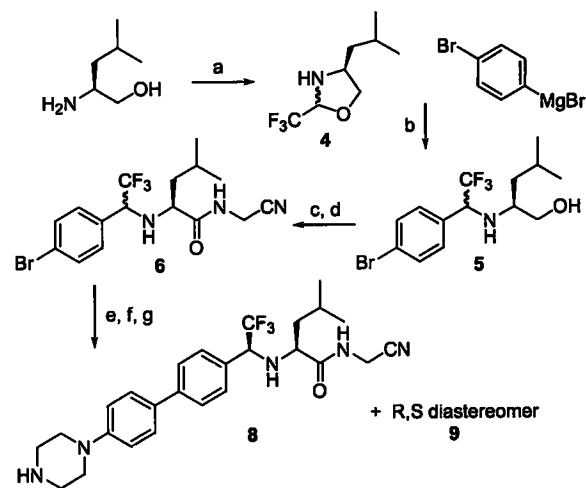
Figure 1. Structures of peptidomimetic cathepsin K inhibitors.

with the NH, while the carbonyl projects into solution.⁶ This implies that the P2-P3 amide bond might be replaced with an isostere without dramatic loss of potency. Our first attempt at such a replacement was to introduce a phenyl ring in place of the amide.⁷ The resulting compound **2** had modest potency but excellent pharmacokinetics, justifying further pursuit of amide replacements. The potency of these compounds could be improved by using an aminothiophene group as in compound **3** to take advantage of the hydrogen bond acceptor at Gly66.⁸ Other methods of introducing the H-bond (alcohols, sulfonamides) were attempted without success.

In the context of developing the piperazine SAR of the aminothiophene derivatives, we found that an *N*-trifluoroethyl group resulted in a dramatic decrease in the basicity of the piperazine. It became quickly apparent that this could be a method for replacing an amide carbonyl, while maintaining a neutral nitrogen H-bond.

The prototype molecule **6** was prepared by condensation of trifluoroacetaldehyde with leucinol to form oxazoline **4** as a 3:1 mixture of diastereomers.⁹ This oxazoline was added to three equiv of 4-bromophenyl magnesium bromide to give trifluoroethylamino alcohol **5** as a 1:1 mixture of diastereomers. More recently, an efficient diastereoselective method for the synthesis of this type of molecule has appeared.¹⁰ Alcohol **5** was oxidized to the carboxylic acid using periodic acid catalyzed by chromic acid.¹¹ Coupling of the aminoacetonitrile was carried out using conventional methods to give **6** (Scheme 1). Trifluoroethylamine **6** behaved as a neutral molecule on TLC ($R_f = 0.40$, 40% EtOAc/1% HOAc/hexanes) and could not be extracted from ethyl acetate with dilute acid. Titration against Cat K showed that **6** was 5-fold more potent than the corresponding dipeptide derivative **7**, even as a 1:1 mixture of diastereomers (Table 1).

Previous work in other series had shown that a phenyl-piperazine provides optimal binding in S3.⁷ This P3 fragment was added to **6** using a Suzuki coupling, as previously described.⁸ The resulting compound was separated by HPLC into its pure diastereomers **8** and **9**. Compound **8** has a Cat K IC_{50} of ≤ 5 pM, the most potent compound ever obtained in this program. Only an upper bound for this IC_{50} could be determined due to limitations of the assay.¹² Diastereomer **9** was 1000-fold less potent in this assay with poor selectivity over Cat L.



Scheme 1. Reagents and conditions: (a) trifluoroacetaldehyde ethyl hemiacetal, benzene, Dean-Stark, 18 h; (b) THF, 0 °C, 1 h; (c) H_2IO_6 (3 equiv), CrO_3 , CH_3CN , 2 h; (d) PyBOP, aminoacetonitrile $\cdot$ HCl, Et_3N , DMF, 14 h; (e) BocPipPhB(OH)₂, $PdCl_2dppf$, Na_2CO_3 , DMF, 90 °C, 6 h; (f) MeOH, THF, 14 h; (g) HPLC separation.

Table 1. Potency of inhibitors in purified enzyme assays

Compound	IC_{50} (nM) ^a			
	Cat K ^b	Cat B ^c	Cat L ^c	Cat S ^c
2	11	3950	3725	2010
3	1.0	123	352	102
6	1.3	3353	34	121
7	7.3	2477	84	145
8	$\leq 0.005^d$	1111	47	451
9	4.6	>10,000	68	902
10	0.015 ^d	344	26	120
12	0.6	1050	3903	>10,000
13	5.0	>10,000	>10,000	>10,000
14	0.4	614	2274	>10,000

^a IC_{50} s are an average of at least two independent titrations. See Ref 15 for assay conditions.

^b Humanized rabbit enzyme.

^c Human enzyme.

^d Low enzyme conditions—see Ref 12.

A direct comparison of **8** to the corresponding dipeptide **10** (not shown) has demonstrated that both potency and selectivity were increased by replacing the amide bond by a trifluoroethylamine moiety (Table 1).

To determine the stereochemistry of the active diastereomer, we turned to X-ray crystallography. The diastereomer of intermediate **5**, which led to the active compounds, was derivatized as an imidazole carboxylic ester **11**. A single crystal X-ray structure revealed that the CF_3 center was in the *S* configuration (Fig. 2).¹³

In the dipeptide nitrile series of inhibitors, the optimal P2 substituent had been found to be a 1,1-cyclohexyl group, as exemplified by **12** (L-006235/CRA-013783).⁵ When this P2 group was used to replace leucine in the trifluoroethylamine compounds, the resulting compound **13** (Fig. 3) was found to be 1000-fold less active

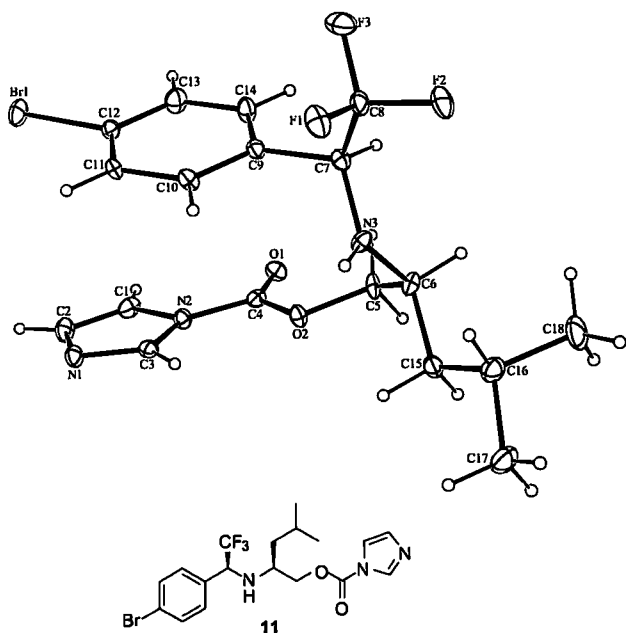


Figure 2. ORTEP representation of **11**. Non-hydrogen atoms are represented by ellipsoids corresponding to 30% probability. Hydrogen atoms have been drawn at an arbitrary size.

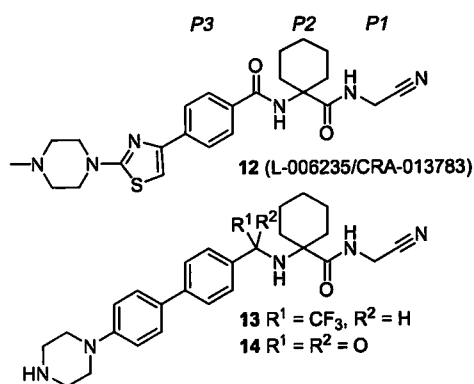


Figure 3. Optimized dipeptide inhibitor L-006235/CRA-013783 and P2-cyclohexyl derivatives **13** and **14**.

than the corresponding amide derivative **14**. Molecular modeling analysis indicated that a steric clash between the CF_3 group and the cyclohexyl ring prevents the molecule from adopting the conformation required for binding. This suggests that only a single substituent will be tolerated at P2 when a CF_3 group replaces the amide carbonyl.

The role of the CF_3 group was explored in a brief SAR study (Table 2). No substitution (**15**) or a simple methyl substituent at this position (**16**) resulted in a dramatic loss of activity. Pentafluoroethyl (**17**) gave similar potency to CF_3 . Aromatic substituents were generally quite potent, as illustrated by the phenyl sulfone substituent (**18**). With the exception of the hydrogen and methyl cases, these amine derivatives tended to have neutral properties, as determined by their non-polar behavior on TLC analysis. This behavior was qualitatively

Table 2. Comparison of CF_3 replacements

Compound	IC_{50} (nM)			
	R	Cat K ^a	R_f ^b	pK_a ^c
6	CF_3	0.9	0.40	1.3
15	H	802	0.05	6.7
16	CH_3	988	0.03	6.7
17	CF_2CF_3	2.4	0.42	1.8
18	4-MeSO ₂ Ph	2.5	0.38	4.6
19	CN	30	0.67	0.7

^a Humanized rabbit enzyme.

^b TLC on SiO₂ plates with 40% EtOAc/1% HOAc/hexanes.

^c Calculated using ACD/ pK_a .

captured by the pK_a of the amine, as calculated by ACD/ pK_a .¹⁴ Most interestingly, a nitrile substituent (**19**), which also gave a non-basic compound, showed a 30-fold loss in activity, implying that the substituent effect was more complex than simply neutralizing the amine.

Compound **6** has been modeled into a Cat K crystal structure (Fig. 4). This model shows that the CF_3 group does not form any lipophilic interactions with the enzyme, but rather is directed away from the active site into water. The hydrogen bond closely mimics the hydrogen bond of the amide, but due to the sp^3 hybridization of the nitrogen, it is oriented better toward the Gly66 carbonyl, potentially forming a stronger hydrogen bond. The aromatic substituent is situated over the glycine shelf in essentially the identical orientation as the aromatic group of the dipeptide inhibitors.

The sum of the above data allows us to propose a rationale for the increased potency of the trifluoroethylamine,

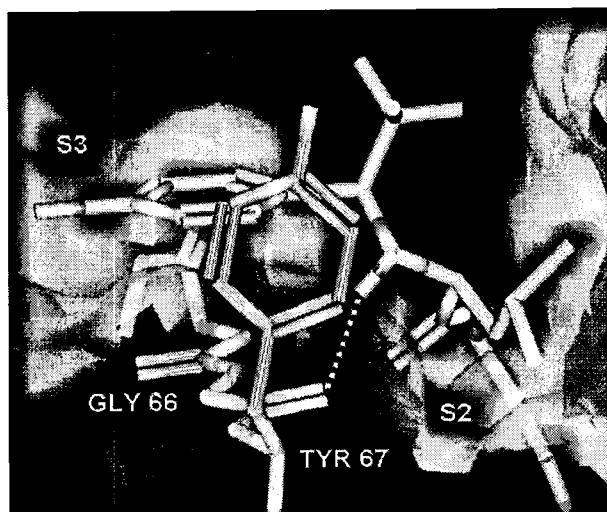


Figure 4. Compound **6** modeled into the active site of human Cat K (pdb entry 1MEM) showing the interaction of the P2-P3 trifluoroethylamine moiety.

relative to the amide. The sp^2 atoms of the amide impose a suboptimal conformational restraint on the aromatic ring and the hydrogen bond. In contrast, the CF_3 group attached to an sp^3 carbon orients itself perpendicular to the aromatic ring and stabilizes the aromatic ring in its bioactive conformation. The sp^3 hybridized nitrogen allows the simultaneous formation of an optimal hydrogen bond. In the case of compound 19, the nitrile substituent is considerably smaller than the CF_3 group resulting in a low barrier to rotation for the aromatic ring. As long as a substituent in this position is large enough to enforce the conformational preference of the aromatic ring, and can prevent protonation of the amine group at physiological pH, a potent compound will be obtained.

This replacement of an amide with a trifluoroethylamine leads to advantages other than potency and selectivity. In the dipeptide nitriles, while the P2-P3 amide bond was found to be metabolically stable, the P1-P2 amide bond can be hydrolyzed to give the carboxylic acid. This was especially apparent in dipeptide inhibitors containing leucine in P2. Replacing the leucine with cyclohexyl effectively blocked this route of metabolism. When the P2-P3 amide is replaced by the phenyl ring of compound 2, this P1-P2 amide hydrolysis is not observed. Similarly, in the case of trifluoroethylamine derivatives described, we found no evidence of a P1-P2 amide cleavage, even though these compounds contain leucine in P2.

In conclusion, we have found that trifluoroethylamine is an excellent surrogate for the P2 amide bond in the inhibitors of Cat K. Not only does this functional group enhance potency and selectivity over other cathepsins, but the resulting compounds are stable to P1-P2 amide bond cleavage that is observed in analogous dipeptide inhibitors. The fully elaborated inhibitor 8 is a ~ 5 pM inhibitor of Cat K and is $>10,000$ -fold selective over other cathepsins.

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- Inhibitory potencies were determined in a version of the published Cat K assay in which the enzyme concentration was reduced to 10 pM—the lowest enzyme concentration at which activity could be reliably measured. At this concentration, IC_{50} s below 5 pM cannot be determined.
- Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 274083 for 11. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [e-mail: deposit@ccdc.cam.ac.uk].
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Identification of a potent and selective non-basic cathepsin K inhibitor

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Received 2 November 2005; revised 16 December 2005; accepted 20 December 2005
Available online 18 January 2006

Abstract—Based on our previous study with trifluoroethylamine as a P2–P3 amide isostere of cathepsin K inhibitor, further optimization led to identification of compound 22 (L-873724) as a potent and selective non-basic cathepsin K inhibitor. This compound showed excellent pharmacokinetics and efficacy in an ovariectomized (OVX) rhesus monkey model. The volumes of distribution close to unity were consistent with this compound not being lysosomotropic, which is a characteristic of basic cathepsin K inhibitors.
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Cathepsin K (Cat K) is a lysosomal cysteine protease believed to be the principal enzyme responsible for degradation of Type I collagen in osteoclastic bone resorption. Thus, an inhibitor of Cat K may be effective as an anti-resorptive for treatment of osteoporosis.¹ Recently, we have reported trifluoroethylamine as an excellent amide isostere for the P2–P3 amide bond of Cat K inhibitors.² When compared to the corresponding dipeptide inhibitors, the trifluoroethylamine derivatives provided enhanced potency for cathepsin K and increased selectivity over other cathepsins. Compound 1 was identified as a 5 pM inhibitor of Cat K with a selectivity of >10,000-fold over other cathepsins. This compound contains a biphenyl piperazine P3 substituent, which has been found to be critical for both potency and selectivity in other series of Cat K inhibitors.^{3,4} However, we have also noted that basic inhibitors are often lysosomotropic, which results in increased potency in off-target cell-based enzyme occupancy assays.⁵ Indeed,

when compound 1 was tested in the whole cell enzyme occupancy assays,⁶ we observed much greater potency against cathepsins B, L, and S than was observed in the corresponding enzyme assays (Table 1).⁷ Similar potency increases in the functional cathepsin K bone resorption assay^{8,9} using rabbit osteoclasts were not observed.⁵ Therefore, the high selectivity of 1 observed in enzyme assays is reduced when the compound is evaluated in these cell-based assays. This reduction of selectivity could increase the potential for adverse events in clinic due to inhibition of off-target proteases. Nevertheless, the enhanced potency and selectivity provided by the trifluoroethylamine amide isostere offered an opportunity to re-visit SAR at the P3 region. Our goal was to identify a potent, selective, and orally bioavailable Cat K inhibitor containing a non-basic P3 substituent which would be devoid of the lysosomotropic properties observed with basic Cat K inhibitors.

Keywords: Bone resorption; Osteoporosis; Cysteine protease; Cathepsin K; Cathepsin K inhibitor; Non-basic cathepsin K inhibitor; L-873724.

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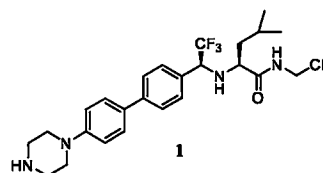
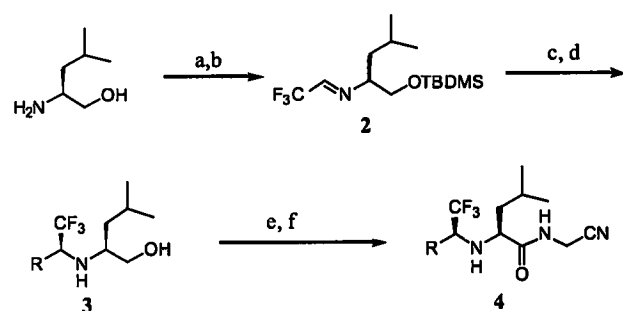


Table 1. Functional, whole cell, and enzyme activity of compound 1

IC ₅₀ (nM)			
Enzyme occupancy assays			Functional Cat K
HepG2 Cat B	HepG2 Cat L	Ramos Cat S	Rabbit bone res
82	16 ^a	7	12 ^a
Enzyme assays			
Cat B	Cat L	Cat S	Rabbit Cat K
1111	47	451	0.01 ^b

^a This value likely represents the lower limit of the assay.^b IC₅₀ was determined at a concentration of 10 pM enzyme.

Our initial synthesis of the trifluoroethylamine moiety was not diastereoselective.² Subsequently an efficient diastereoselective method was developed (Scheme 1). Commercially available (*S*)-leucinol was converted to imine **2** and then reacted with alkyl, aryl, or heteroaryl lithium reagents in a diastereoselective manner.¹⁰ Deprotection of the silyl group gave the desired (*S,S*)-amino alcohol intermediate **3** with diastereoselectivities ranging from 5:1 to 20:1; the accompanying (*R,S*)-diastereomer could be partially removed by chromatography in many cases. Oxidation with periodic acid in the presence of catalytic chromium trioxide¹¹ provided the carboxylic acid intermediate.¹² The diastereomeric puri-



Scheme 1. Reagents and conditions: (a) *tert*-Butyldimethylsilyl chloride, Et₃N, cat. DMAP, CH₂Cl₂, room temperature, 2 h; (b) trifluoroacetaldehyde methyl hemiacetal, benzene, reflux with a Dean–Stark trap, overnight, 85% for two steps; (c) R–Li, –78 °C, 2 h; (d) TBAF, THF, HOAc, room temperature, 2 h, 15–75% for two steps; (e) H₂IO₄/CrO₃, Na₂HPO₄, CH₃CN, 0 °C, 1 h; (f) aminoacetonitrile hydrochloride, HATU, Et₃N, DMF, room temperature, 2 h, 10–55% for two steps.

Table 2. In vitro activity of simple P3 analogs

Compound	R	IC ₅₀ ^a (nM)				
		Cat K ^b	Cat B	Cat L	Cat S	Bone res ^c
8	H	4123	>10,000	>10,000	6183	
9	Butyl	391	>10,000	1950	1308	
10	Phenyl	6.7	>10,000	34	36	200
11	4-Bromophenyl	0.8	2993	13	54	111

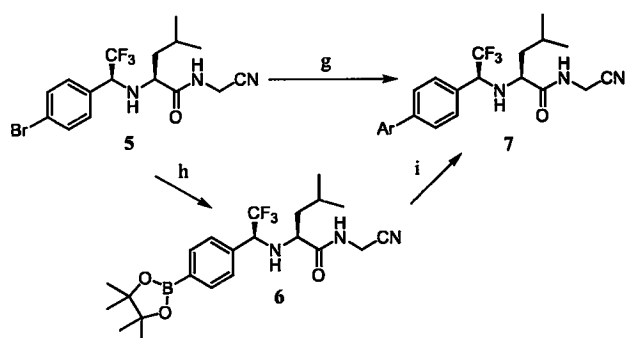
^a IC₅₀s are an average of at least two independent titrations.^b Humanized rabbit enzyme (Ref. 3), see Ref. 6 for assay conditions.^c Rabbit bone resorption assay—functional assay, see Ref. 8 for assay conditions.

ty of this acid could be further improved by trituration in diethyl ether or by crystallization of the dicyclohexylamine salt. Amide bond formation between the acid intermediate and aminoacetonitrile then afforded **4**.¹³

Early in the SAR study, it was quite clear that simple P3 substituents would not provide sufficient potency and selectivity (Table 2). Compound **8** lacking a P3 substituent (R = H) exhibited low potency against all the tested cathepsins. Introduction of a small alkyl substituent such as the butyl group in **9** improved the activity, while the phenyl analog **10** provided a dramatic improvement in potency against cathepsins K, L, and S. The 4-bromo-substituted analog **11** showed a further improvement in potency of Cat K. This beneficial effect of para substitution on the phenyl group of **10** prompted us to pursue our SAR studies in this direction.

Our strategy for obtaining a wide range of 4-substituted analogs was to use rapid analog synthesis based on a Suzuki coupling reaction with readily available boronic acids or arylhalides. For these subsequent studies, the bromide **5** and boronate **6** serve as convenient intermediates for Suzuki coupling reactions to provide biaryl inhibitors as represented by compound **7**. Bromide **5** was obtained from imine **2** as described in Scheme 1, where the lithium reagent used was derived from 1,4-dibromobenzene (Scheme 2). In the cases where the substituent in the Ar group of compound **7** could be further transformed, an appropriate reaction would be used to complete the conversion such as methyl sulfide to methyl sulfone by oxidation reaction.

The results of representative examples are summarized in Table 3. The replacement of the bromo substituent in **11** with a phenyl group (**12**) resulted in the improvement of Cat K potency and selectivity against Cat L and Cat S, and thus confirmed the beneficial effect of para substitution at the proximal phenyl ring. Further substitutions on the distal phenyl ring were generally tolerated. However, there was no clear trend for the SAR on potency and selectivity. Most compounds showed selectivities of >5000-fold for cathepsin B, >1000-fold for cathepsin L, and >500-fold for cathepsin S based on the enzyme assays. Mono-substitution with halo (**13–15**), alkyl (**18**), electron-donating (**19** and **21**),



Scheme 2. Reagents and conditions: (g) ArB(OH)_2 , $\text{PdCl}_2(\text{dppf})$, aq Na_2CO_3 , DMF, 80 °C; (h) bis(pinacolato)diboron, $\text{PdCl}_2(\text{dppf})$, KOAc; (i) Ar-(Br or I) , $\text{PdCl}_2(\text{dppf})$, aq Na_2CO_3 , DMF, 80 °C.

and electron-withdrawing (20, 22, 23 and 24) substituents provided compounds with similar potency against the Cat K enzyme (IC_{50} 0.1–0.9 nM). Disubstitution was also tolerated, but the substitution patterns were critical to potency, as the 3,4-difluoro analog 16 has an IC_{50} of 1.1 nM and the 3,5-difluoro analog 17 is less potent with an IC_{50} of 6 nM against Cat K. Insertion of

a spacer atom either as an ether linker (25) or a carbonyl (26) reduced Cat K potency. Among these biphenyl analogs, the most selective compound appeared to be 13 with >6000-fold selectivity for Cat S and over 16,000-fold for cathepsins B and L. The most potent non-basic biphenyl analog in the rabbit bone resorption assay is the methyl sulfone analog 22. The SAR with heterocyclic substituents was also studied. Some representative examples are also shown in Table 3. Pyridine 27, thiophene 28, thiazole 29, and indole 32 are very potent against Cat K, while oxadiazole 30 and pyrimidine 31 show reduced potency. In the functional rabbit bone resorption assay, the pyridine 27 has excellent potencies, whereas other heterocyclic analogs are less potent. However, pyridine 27 shows poorer selectivity than the biphenyl analog 22 against Cat L (267-fold vs 1320-fold) and Cat S (206-fold vs 890-fold). The tricyclic P3 analogs 33 and 34 were briefly studied. This modification resulted in poor pharmacokinetics in rats and therefore was not pursued further. Nevertheless, the SAR showed increased selectivity over Cat L and S, but decreased selectivity over Cat B (33 vs 22 and 34 vs 27). Among two of the best biphenyl analogs, 13 and 22, preliminary pharmacokinetic study showed that 22 had superior

Table 3. In vitro activity of representative P3 analogs

Compound	R	IC_{50}^a (nM)				
		Cat K ^b	Cat B	Cat L	Cat S	Bone res ^c
1	4-Piperazin-1-yl phenyl	$\leq 0.005^d$	1111	47	451	12 ^e
12	Phenyl	0.2	1605	1473	303	44
13	2-Fluorophenyl	0.1	6086	1670	614	28 [*]
14	3-Fluorophenyl	0.5	2476	2792	278	67 [*]
15	4-Fluorophenyl	0.6	5939	887	449	29 [*]
16	3,4-Difluorophenyl	1.1	8869	1001	189	46 [*]
17	3,5-Difluorophenyl	6	>10,000	3002	249	n.d.
18	4-Methylphenyl	0.3	1337	809	282	27 [*]
19	4-Methoxyphenyl	0.1	1660	560	196	n.d.
20	4-Cyanophenyl	0.9	7392	344	172	30 [*]
21	4-(Methylthio)phenyl	0.2	1400	524	153	50 [*]
22 ^f	4-(Methylsulfonyl)phenyl	0.2	5239	264	178	13 ^e
23	4-(Aminosulfonyl)phenyl	0.2	1561	470	154	52
24	3-(Methylsulfonyl)phenyl	0.3	6984	1480	265	53 [*]
25	Phenoxy	2.3	>10,000	771	220	88 [*]
26	Benzoyl	1.5	>10,000	1915	254	151 [*]
27	Pyridin-4-yl	0.3	2374	80	62	19
28	3-Thienyl	0.2	1155	1475	365	33 [*]
29	1,3-Thiazol-2-yl	0.3	4818	878	240	52
30	3-Methyl-1,2,4-oxadiazol-5-yl	2.6	4073	314	436	118 [*]
31	Pyrimidin-2-yl	44	5903	1857	1698	93 [*]
32	4-(1 <i>H</i> -Indol-4-yl)	0.4	4699	3047	607	80 [*]
33	4'-(Methylsulfonyl)biphenyl-4-yl	1.2	1873	2422	653	5 ^e
34	4-Pyridin-4-yl-phenyl	<0.2	483	342	188	14 ^{e,*}

^a IC_{50} s are an average of at least two independent titrations unless specified otherwise.

^b Humanized rabbit enzyme (Ref. 3), see Ref. 6 for assay conditions.

^c Rabbit bone resorption assay, see Ref. 8 for assay conditions.

^d IC_{50} was determined at a concentration of 10 pM enzyme.

^e This value likely represents the lower limit of the assay.

^f L-873724.

^{*} $n = 1$. n.d. = not determined.

pharmacokinetics to **13** in rats. Therefore, compound **22**, now designated as L-873724, was selected for further profiling.

L-873724 has good pharmacokinetics in three preclinical animal species (Table 4). The volumes of distribution close to unity are consistent with this compound not being lysosomotropic. In the whole cell enzyme occupancy assays (Table 5), L-873724 showed no significant increases in potency against Cat B, L, and S compared to isolated enzyme potencies, further evidence supporting its non-lysosomotropic character. In addition, L-873724 had excellent potency in the rabbit bone resorption assay. As a result, L-873724 is anticipated to maintain good functional selectivity in vivo.

The important question of whether a non-basic inhibitor L-873724 would show efficacy in an animal model of bone resorption now needed to be addressed. The possibility exists that the lysosomotropic properties associated with basic Cat K inhibitors may help the accumulation of compound in the acidic osteoclast resorption lacunae where cathepsin K digests collagen and therefore contribute to efficacy. In this context, one could conclude that a non-basic inhibitor would show reduced efficacy in vivo. Fortunately, in the ovariectomized (OVX) rhesus monkey model,¹⁴ a mean decrease of 68% in urinary uNTx/Cr was observed during the six-day on-treatment phase at 3 mg/kg with once-daily oral dosing, as indicated in Figure 1. Three days following the last dose, uNTx/Cr levels returned to baseline. The response of L-873724 in this model is comparable to that of the basic P2–P3 amide dipeptide Cat K inhibitor CRA-013783/L-006235 which has similar in vitro potency.¹⁵

An X-ray crystallographic structure of compound **23**, which is a close analog of **22**, with Cat K at 2 Å resolution has been obtained (Fig. 2, PDB entry 2FD2). Our previous model structure² of trifluoroamine analog into

Urine NTx/Cr during 3mpk L-873724 po, qd
Adult OVX Rhesus Monkeys

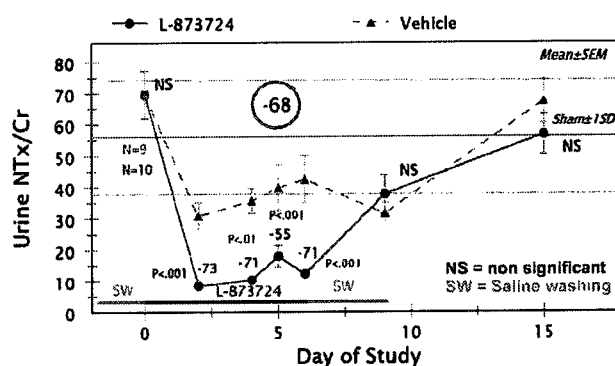


Figure 1. Efficacy of compound **22** (L-873724) in the OVX rhesus monkey model.

Cat K shows excellent agreement with the X-ray crystal structure. The CF₃ group can clearly be seen directed out of the active site, toward water. The hydrogen bond between the NH adjacent to the CF₃ group and the Gly66 carbonyl is well formed and closely resembles the comparable hydrogen bond found in the P2–P3 amide dipeptide series. The phenyl group adjacent to the CF₃ group is coplanar with the glycine shelf (Gly66–Gly65) creating a significant hydrophobic interaction. Due to the sp³ nature of the trifluoroethylamine group, the orientations of phenyl ring over the glycine shelf and the orientation of the hydrogen bond are preferred. This is in contrast to the P2–P3 amide dipeptide inhibitors where both of these interactions are suboptimal due to the sp² nature of the linking amide bond. The sulfonamide substituent at the distal phenyl ring is largely exposed outside the binding pocket and does not have significant interaction at the active site. In addition, one side of the distal phenyl ring is also exposed to solvent and the other side is in the S3 region of the enzyme. This may help to explain why the substi-

Table 4. Pharmacokinetics of **22** (1% methocel as dosing vehicle)

Species	po dose (mg/kg)	% F	C _{max} (μM)	t _{1/2} (h)	V _d (L/kg)
Rat	10	40	4.4	3	1.4
Dog	10	65	18	6	0.8
Rhesus monkey	5	24	0.7	2	0.5

Table 5. Functional, whole cell, and enzyme activity of compound **22**

IC ₅₀ (nM)			
Enzyme occupancy assays			Functional Cat K
HepG2 Cat B	HepG2 Cat L	Ramos Cat S	Rabbit bone res
4807	1221	95	13 ^a
Enzyme assays			
Cat B	Cat L	Cat S	Rabbit Cat K
5239	264	178	0.8 ^b

^a This value likely represents the lower limit of the assay.

^b IC₅₀ was determined at a concentration of 10 pM enzyme.

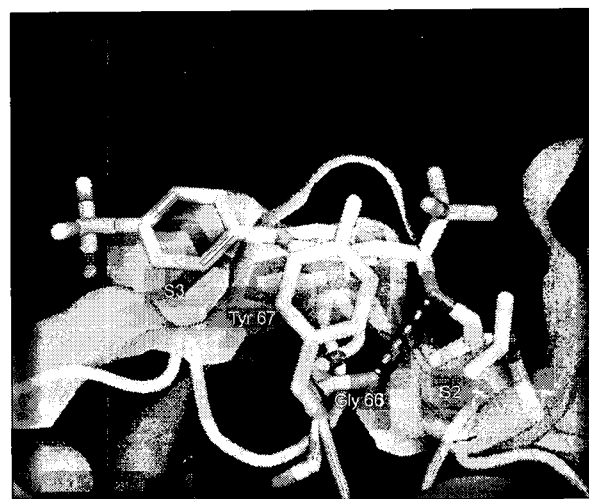


Figure 2. X-ray crystal structure of compound **23** bound to Cat K showing the P2–P3 trifluoroethylamine group and the hydrogen bond it makes to Gly66 (PDB entry 2FDZ).

tution pattern of disubstituted analogs is critical for potency, **16** versus **17**. Overall the binding interactions with compound **23** are essentially identical to that of the P2–P3 amide dipeptide inhibitors (pdb entry 1MEM), without the internal destabilization of the ligand found in the dipeptides.

In summary, we have identified L-873724 as a non-basic, potent, and highly selective cathepsin K inhibitor. This compound is orally bioavailable, non-lysosomotropic, and efficacious in the rhesus monkey bone resorption model.

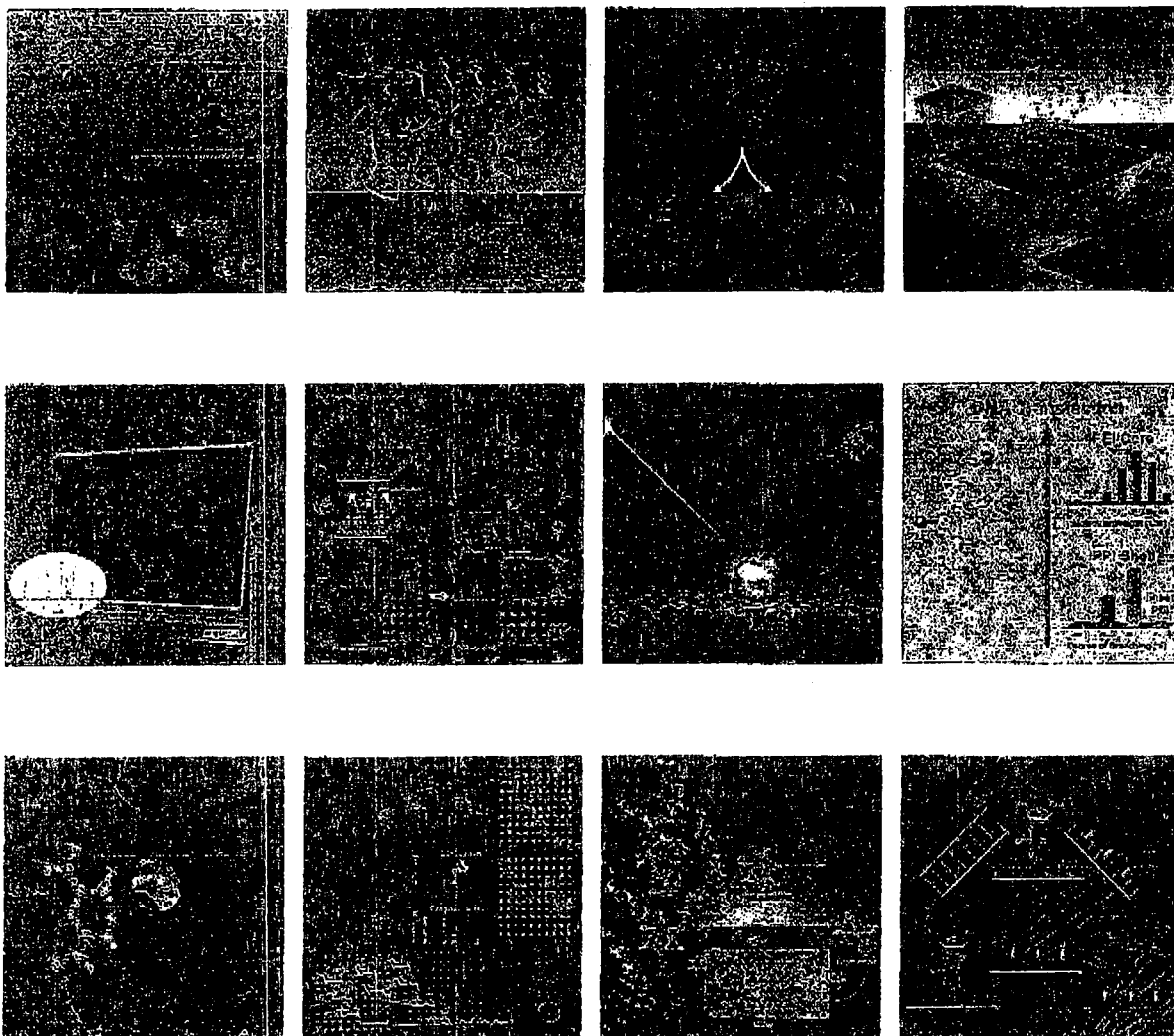
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A EUROPEAN JOURNAL

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Chemistry & *Life* Sciences

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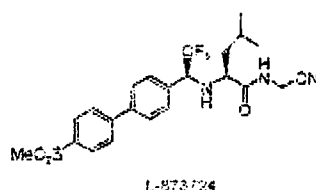
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Table of Contents

W. C. Black,* M. D. Percival

1525 – 1535

The Consequences of
Lysosomotropism on the Design of
Selective Cathepsin K Inhibitors



Better with fluorine. Basic cathepsin K inhibitors accumulate in the lysosomes of cells, impairing selectivity against off-target lysosomal cathepsins as measured by whole-cell enzyme-occupancy assays. Replacing an amide bond in these inhibitors with a trifluoroethylamine group increases the potency and selectivity enough to allow removal of the basic substituent and provides inhibitors such as L-873724 with high cellular selectivity and in vivo efficacy.

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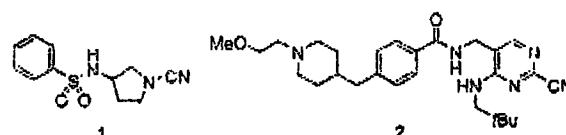
Certain types of electrophiles, such as epoxides, vinyl sulfones, fluoromethyl ketones, and diazomethylketones, lead to irreversible covalent modification of the protease. Such inhibitors could pose significant safety concerns as drug candidates, but make excellent tools for identifying and understanding the roles of cysteine proteases. Other classes of electrophiles such as aldehydes, nitriles, and ketones without adjacent leaving groups trap the nucleophilic cysteine in a covalent but reversible fashion. Ketones have been perhaps the most thoroughly studied. A wide variety of structures are possible by varying the nature of the substituent on the amino side ("prime side") of the ketone. This provides the ability to modulate the electrophilicity of the carbonyl group. Additionally, it is possible to take advantage of additional binding interactions in the S1' pocket of the enzyme active site. These advantages are often countered by the poor pharmacokinetic properties associated with the large size of the resulting inhibitors and the configurational lability of stereocentres adjacent to the ketone moiety.

Nitrile-containing molecules have been known to inhibit cysteine proteases since the late 1960s when benzamidoacetone nitrile was shown to be a 400 μM inhibitor of papain.^[20] Addition of the active-site cysteine to the nitrile leads to the formation of a thioimide in which the nitrogen is protonated by the active-site histidine residue.^[21]

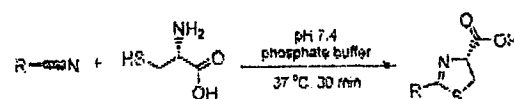
Simple benzo- or alkynitriles make rather poor electrophiles, but this electrophilicity can be modified significantly by the introduction of electron-withdrawing groups. Cyanamides are particularly reactive, and even small molecules containing this electrophile, such as **1** (Scheme 1), show significant activity in purified cathepsin assays. ¹³C NMR experiments on **1** in the presence of papain, have shown the formation of a reversible isothiourea adduct with the active-site cysteine.^[22] However, this reactivity leads to unacceptable levels of irreversible covalent binding (552 pmolequiv per mg after 1 h of incubation with human liver microsomes) even in the absence of NADPH. Irreversible covalent binding has been correlated to haptenization resulting in an immunological response, such that Merck has set a threshold of 50 pmolequiv per mg protein for development candidates.^[22] With covalent binding eleven times above this threshold, cyanamides would probably make poor drug candidates. Similarly, the electrophilicity of a benzonitrile is dramatically increased by incorporating nitrogen atoms into the aromatic ring to provide cyanopyridines or cyanopyrimidines (e.g., **2**)^[24] In this structural class, cathepsin K potency tracks with the electrophilicity of the nitrile war-

head. Additionally, compound **2** was found to have high levels of irreversible protein labelling (1665 pmolequiv mg⁻¹ after 1 h of incubation with human liver microsomes—S. Day, unpublished results). The α -amidoacetone nitrile group appears to have an optimal level of electrophilicity: little reactivity is observed with sulfur nucleophiles such as glutathione, yet potent inhibition of cathepsins can still be achieved.

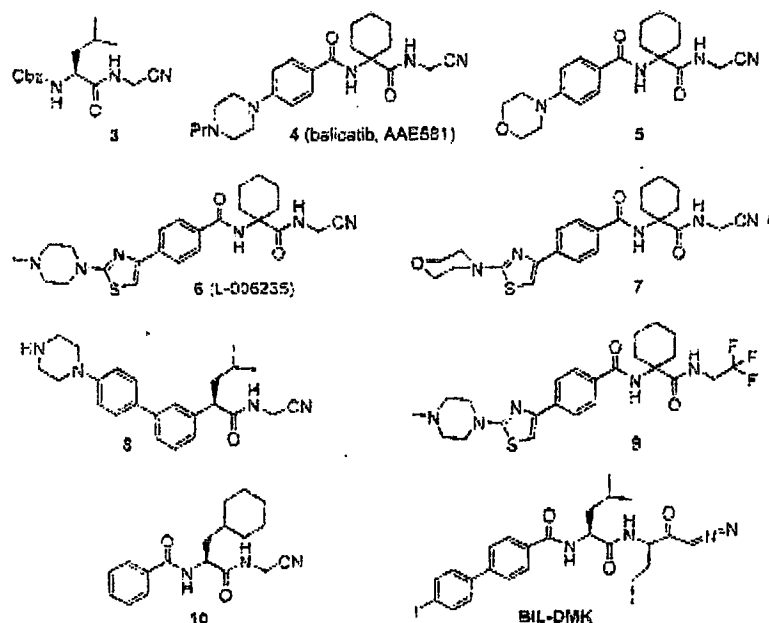
An objective measure of nitrile electrophilicity can be obtained by incubating compounds with cysteine (3 mM in pH 7.4 phosphate buffer at 37 °C for 30 min). The resulting thiazoline products (Scheme 2) can be quantified by LC-MS-MS analysis in comparison to synthetic standards to provide a measure of chemical reactivity. In this assay, cyanamide **1** and cyanopyrimidine **2** show 71% and 79% conversion to the cysteine adduct, respectively. In contrast, the α -amidoacetone nitrile derivatives **4** and **6** (Scheme 3) give only 9% and 4% conversion to the cysteine adduct reactivity, respectively (R. Oballa,



Scheme 1. Cathepsin inhibitors with reactive nitrile electrophiles.



Scheme 2. Conversion of nitrile to thiazoline to assess electrophilicity.



Scheme 3. Structures of cathepsin inhibitors.

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unpublished results). The low level of cysteine adduct formation in 6 correlated well with the observed covalent binding of 10 pmolequiv⁻¹h⁻¹ in human liver microsomes.

The reversibility of the inhibition of cathepsin K and other cysteine cathepsins by α -amidoacetonitrile inhibitors can often be demonstrated by dilution of a preformed enzyme-inhibitor complex into substrate-containing buffer and following the recovery of enzyme activity (Figure 1).^[22] Such experiments re-

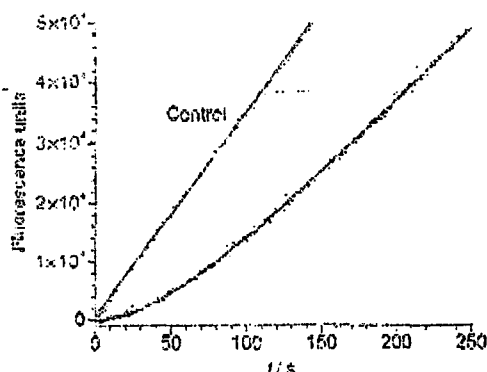


Figure 1. Progress curve showing the reversibility of cathepsin K inhibition by 6. Human cathepsin K (5 nM) was incubated with 5 nM 6 for 30 min prior to a 100-fold dilution into substrate (2 μ M Z-Leu-Arg-AMC)-containing pH 5.5 buffer. The data were fitted to an equation describing a first-order recovery of activity. The control represents cathepsin K pretreated with vehicle (DMSO) prior to dilution.^[22] (J.-P. Falgueyret, unpublished results).

quire that the resulting enzyme concentration is below the inhibitor K_d , or little recovery of activity will be observed. Although the inhibition of cathepsin K by α -amidoacetonitrile inhibitors is reversible, the predicted thioimide covalent complex can be trapped by rapid denaturation with organic solvent (Figure 2).

The optimization of α -amidoacetonitrile-containing cathepsin K inhibitors has been largely carried out by using a dipeptide template, as illustrated by compound 3 (Scheme 3). The facile synthesis of peptide analogues has allowed the structure-activity relationship (SAR) of the active site to be optimized in rapid fashion. This approach has been guided by the availability of high-resolution crystal structures of inhibitors covalently bound to cathepsin K. These structures show a relatively small, lipophilic S2 pocket and a large, shallow S3 subsite featuring an exposed aspartic acid residue.^[23] Peptide inhibitors form hydrogen bonds from both the carbonyl and NH of the P1 amide, and an important hydrogen bond from the NH of the P2 amide. An amino acid scan of P2 showed that leucine was preferred in this position.^[26] A 1,1'-cyclohexyl ring in this position provides similar potency and has the advantages of increased selectivity over other cathepsins and reduced hydrolysis of the P1 amide bond.

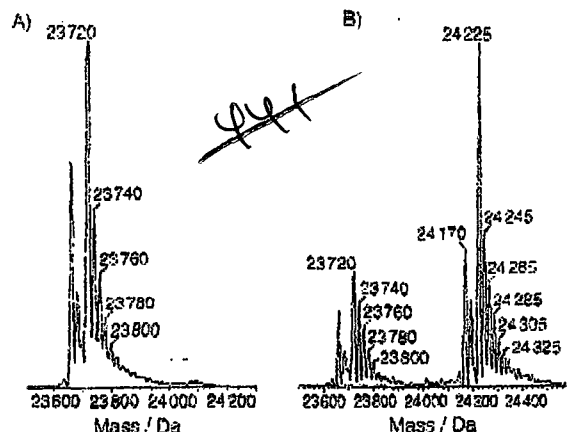


Figure 2. LC-MS (QTOF) spectrum of a trapped cathepsin K- α -amidoacetonitrile inhibitor thioimide complex. A) The spectrum of native cathepsin K. B) The spectrum of a 1:1 molar mixture (2 μ M) of cathepsin K and α -amidoacetonitrile inhibitor (molecular mass 507.6) incubated in pH 5.5 assay buffer for 60 min and then rapidly denatured by the addition of 1 volume acetonitrile. The mass difference between the two mass envelopes of panel B (505 Da) corresponds to the expected mass increase resulting from the formation of a cathepsin K-thioimide complex (506.6 Da).^[14,26] To demonstrate the reversibility of the α -amidoacetonitrile inhibitor under these buffer conditions, the cathepsin K-inhibitor complex was also treated with 100 μ M E64 for 24 h, prior to denaturation and analysis by LC-MS (QTOF, Micromass). The resulting protein mass was consistent with the quantitative formation of the E64 adduct (J.-P. Falgueyret and T. LeRiche, unpublished results).

The SAR of P3 showed a marked preference for an aromatic group attached to the amide carbonyl. X-ray crystallography shows this ring sitting above the π -face of a Gly-Gly sequence ("glycine shelf"). Substituting this aromatic ring with a basic group provides an increase in both potency and selectivity. This effect, which has been attributed to a positive ionic interaction with the P3 aspartic acid residue (Asp61), is not available in off-target cathepsins. A wide variety of linkers between the aromatic ring and the basic nitrogen are tolerated. A piperazine directly attached to the phenyl group gives 4 (AAE581, balicatib), a 1.4 nM human cathepsin K inhibitor being developed by Novartis.^[27] The addition of an intervening thiazole between the phenyl ring and the piperazine gives 6, (CRA-013783, L-006235), a 0.2 nM inhibitor of human cathepsin K.^[26] A direct comparison of 4 and 6 to their nonbasic morpholine analogues 5 and 7 illustrates the value of the basic P3 substituent (Table 1). The piperazines provide a 25-fold increase in cathepsin K potency relative to the corresponding morpholines

Cmpd	Enzyme IC ₅₀ [nM]				Rat pharmacokinetics	
	Cat K	Cat B	Cat L	Cat S	$V_{0.5}$ [L kg ⁻¹]	$t_{1/2}$ [h]
4	1.4	2 900	18 000	26 000	4.7	2.9
5	43	15 210	32 570	51 630	1.9	0.9
6	0.2	895	2 750	> 10 000	11	4.0
7	5.5	2 700	5 900	> 10 000	0.8	0.3

with little effect on the closely related cysteine cathepsins B, L, or S. The pharmacokinetic parameters of the basic and nonbasic analogues differ as well. The piperazines 4 and 6 have large volumes of distribution with terminal half-lives that are markedly longer than those of the morpholines. Since several CYPs, including CYP3A, are known to preferentially recognize hydrophobic substrates,^[22] it is possible that the charged piperazine protects the inhibitor from CYP-mediated metabolism relative to the morpholine, leading to a longer half-life.

Activity-Based Probes to Measure Cysteine Cathepsin Activity

Activity-based probes (ABPs) are electrophile-containing inhibitors that irreversibly inactivate their target enzymes by reaction with an active-site residue that is critical for enzyme activity.^[29] In general, ABPs contain three distinct moieties; the electrophilic "warhead", a linker region that may increase the affinity for the target enzyme and provide the desired target specificity and a tag that is used to identify the labelled protein. This tag may, for example, be a radionuclide, stable isotope, biotin or a fluorescent dye.^[30–32] An advantage of this technique is that the activity of individual proteases can be estimated in intact cells and tissues, without the requirement for specific substrates for each enzyme of interest. Most recently, quenched ABPs have been developed that become fluorescent upon activity-dependent covalent modification of the target protease.^[33]

Several ABPs have been described for cysteine cathepsins. The epoxysuccinyl and diazomethylketone (DMK) warhead-containing ABPs generally show selectivity for cysteine proteases over other protease families.^[33,34,35] We have focused on DMK-containing ABPs, in particular ¹²⁵I-BIL-DMK (Scheme 3) to follow cysteine cathepsin activity in cells and to identify cell-based assays capable of determining the potencies of competitive, reversible inhibitors against the ABP target cathepsins. The basis of these cell-based assays, which are termed enzyme-occupancy assays, is the intracellular competition for the target enzyme active site between the reversible inhibitor and the irreversible ABP. To quantitate ABP labelling, the labelled proteins are separated by 1D or 2D gel electrophoresis

and subjected to autoradiography. When the assay is run under appropriate conditions, the IC₅₀ value for the inhibition of ABP labelling can give useful information on the cellular permeability of the reversible inhibitor and its affinity for the cathepsins present in their native environment in the cell. Clearly, the use of a single ABP, which is potent against the target (cathepsin K) and off-target enzymes (cathepsin B, L, S) would simplify the development of these assays, rather than using a separate ABP for each enzyme. BIL-DMK is a relatively potent inactivator ($k_{\text{obs}}/[\text{I}] > 2.6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) of the endoprotease cathepsins B, K, L, S, F, and V with apparent IC₅₀ values of less than 100 nM under the assay conditions of Table 2.^[34]

Table 2. Inhibition of endogenous BIL-DMK against various cysteine cathepsins			
	IC ₅₀ (nM)		IC ₅₀ (nM)
cathepsin B	62	rabbit cathepsin K	2.1
cathepsin L	8.9	cathepsin S	10
cathepsin C	870	cathepsin F	10
cathepsin H	5270	cathepsin V	2.2
cathepsin Z	> 10000		

[a] Enzyme, inhibitor preincubation time was 2 min, prior to addition of substrate.

For an ABP to be practically useful, it must be reactive towards a relatively small number of targets from which it must also be readily separable. These criteria were met as ¹²⁵I-BIL-DMK specifically labelled only mature, active forms of cathepsins B, L, S, and K when incubated at a concentration of 1 nM for 60 min with HepG2, Ramos and rabbit synovocyte (HIG82) cells, respectively. Following separation on 2D gels, the radioactive spots were positively identified by a combination of predicted molecular mass, pI (isoelectric point), and comigration with immunoreactive species by Western blotting (Figure 3).^[34]

Fortunately, the species labelled by ¹²⁵I-BIL-DMK were also well separated when run on 1D SDS-PAGE. The optimal conditions for the whole-cell enzyme-occupancy assay with HepG2, Ramos and HIG82 cells require a 60 min preincubation of cells with reversible test inhibitor to allow penetration of the cell

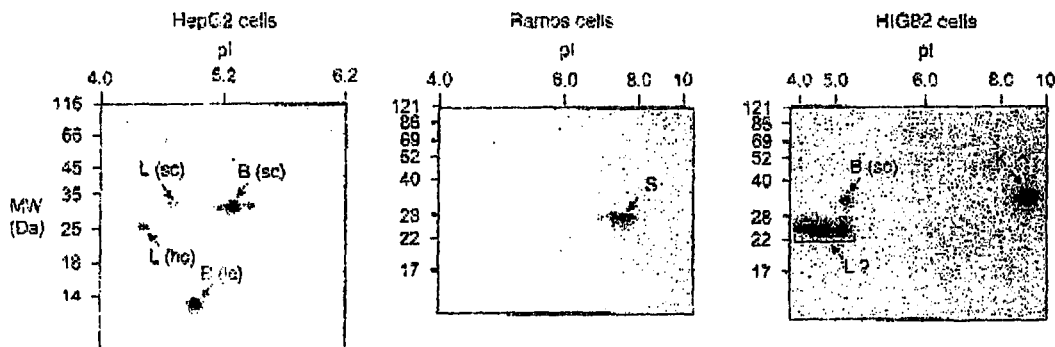


Figure 3. Autoradiographs of 2D gels from intact HepG2, Ramos and HIG82 cells treated with 1 nM [¹²⁵I]-BIL-DMK for 60 min. Letters refer to cathepsin identification, sc: single-chain form, hc: heavy chain, lc: light chain.

and establishment of an equilibrium between the inhibitor and each cathepsin to which it binds. This is followed by the addition of 1 nM [¹²⁵I]-BIL-DMK and incubation for a further 30 min, during which time cathepsin labelling occurs. The reaction is quenched by the addition of a large excess of cold BIL-DMK prior to cell lysis. The labelled species are then separated by 1D SDS-PAGE, and each band is quantified by autoradiography to give an IC₅₀ value for each labelled band (Figure 4).^[24]

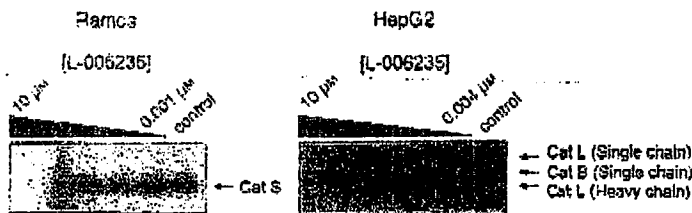


Figure 4. Autoradiographs of 1D gels from intact Ramos and HepG2 cells incubated with 0–10 μM **6** for 60 min, followed by 1 nM [¹²⁵I]-BIL-DMK for 30 min. Cells were then treated with 10 μM cold BIL-DMK, lysed and separated by SDS-PAGE.

For the results of this assay to be meaningful, it is essential that the appropriate ABP concentration and incubation time are chosen. A high ABP concentration and/or long incubation time would result in the displacement of the reversible test compound, causing an underestimation of inhibitor potency. To ensure that this was not the case, experiments were performed at shorter, as well as longer incubation times with the probe. No significant differences in inhibitor IC₅₀ values were observed unless the ABP incubation time was increased to 90 min. The intra- or extracellular localization of the cathepsins labelled by [¹²⁵I]-BIL-DMK in the three cell types was determined by using pairs of cell-permeable and non-cell-permeable inhibitors. In each case, the cell-permeable inhibitors (E64d and CA074Me) competed with the [¹²⁵I]-BIL-DMK labelling of cathepsins B, K, L, and S, whereas their non cell-permeable analogues (E64 and CA074) showed no effect at concentrations of up to 10 μM.^[24]

The enzyme-occupancy assays described above were used, in combination with the functional rabbit osteoclast bone-resorption assay, to define the cellular selectivity of cathepsin K inhibitors against cathepsins B, L, and S.^[24] A review of the data obtained showed that many compounds appeared more

potent in the enzyme-occupancy assays than would have been predicted on the basis of their purified enzyme IC₅₀ values. In contrast, a subset of compounds was not shifted between these cell and enzyme assays. A comparison of the structures of these inhibitors showed that those that appeared more potent had a basic-nitrogen-containing P3 moiety. To illustrate this point, the potencies of compounds 4–8 against cathepsins B, L, and S in enzyme-occupancy assays (in HepG2 and Ramos cells) are shown in Table 3. The basic-nitrogen-containing 4, 6, and 8 are of the order of ten- to 80-fold more potent in the cell-based enzyme-occupancy assays compared to the corresponding purified-enzyme assays. In contrast, the cathepsin B, L, and S potencies of the nonbasic 5 and 7 are similar in both assays. The same effect was also detected for cathepsin K, as the potency of the basic 8 was 17-fold greater in the rabbit synoviocyte (HIG82 cell) enzyme-occupancy assay than against purified rabbit cathepsin K. Small increases in potencies were also observed for other basic compounds such as 4 and 6 in this whole-cell enzyme-occupancy assay over the purified-enzyme assay. The smaller magnitude of these shifts probably reflects the lower limit of the cell-based assay of approximately 1 nM and the fact that these compounds are highly potent against cathepsin K. No systematic shifts in potencies between the cathepsin K enzyme and cell-based assays were found for nonbasic compounds.^[24]

Lyssosomotropism of Cathepsin Inhibitors

The increased inhibitor potencies of basic compounds in the cell-based enzyme-occupancy assays compared to those in purified-enzyme assays was attributed to the compounds' lysosomotropic properties. Lyssosomotropism is the ability of weakly basic, lipophilic molecules to accumulate in acidic compartments.^[27] The neutral species can diffuse across the cell membrane, but becomes membrane impermeable once protonated within the acidic compartment. The degree of compound accumulation, which can reach several hundred fold, is dependent on compound lipophilicity, pK_s, and the pH difference between the cytosol and the lysosome/endosome.^[28] As the target and antitarget cathepsins are localized in the acidic lysosome/endosome, the effective inhibitor concentration encoun-

Table 3. Potencies of basic and nonbasic cathepsin K inhibitors against rabbit cathepsin K and human cathepsins B, L, and S in purified-enzyme and cell-based enzyme-occupancy assays								
Rabbit cathepsin K			Inhibition of cathepsins, IC ₅₀ (nM)					
Enzyme		Cell (HIG82)	Human cathepsin B		Human cathepsin L		Human cathepsin S	
			Enzyme	Cell (HepG2)	Enzyme	Cell (HepG2)	Enzyme	Cell (Ramos)
4	5	1	4800	61	503	48	65000	2900
5	–	–	15400	4400	32000	> 10000	52000	4800
6	1.5	0.75	1100	17	6300	340	47000	790
7	10	5	4200	2900	24000	> 10000	27000	> 10000
8	33	2	35000	4400	2600	320	20000	1340
20	0.01	0.7	1111	82	47	16	451	7
26	0.6	1.5	5239	4807	264	1221	178	95

tered by the enzyme is increased relative to that outside the acidic compartment. The distal P3 nitrogen atoms of compounds 4, 6, and 8 have pK_a values of 7.0–8.8, and the molecules have $\log P$ values of 1.8–3.5; this is consistent with lysosomotropic behaviour.^[36]

Further studies were consistent with this lysosomotropism hypothesis. The piperazine-thiazole-phenyl group of 6 is intrinsically fluorescent; this enabled its subcellular distribution to be determined in whole live cells by two-photon confocal microscopy. When treated with 10 μ M 6, HepG2 cells show a punctated cytoplasmic fluorescence (Figure 5A), whereas vehi-

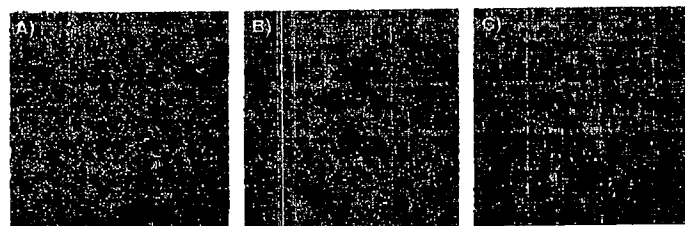


Figure 5. Accumulation of 6 and 9 within the lysosomes of HepG2 cells as observed by two-photon confocal microscopy. HepG2 cells were incubated for 1 h with 10 μ M of either A) 6, B) 9 or C) DMSO vehicle (all measured at an original magnification of 20 $\times$).

cle-treated cells have little fluorescence (Figure 5C). This cellular fluorescence was blocked when the cells were pretreated with NH_4Cl , which causes the disruption of the cytoplasmic-lysosomal pH gradient. A similar pattern of cellular fluorescence was obtained with the analogue 9 (Scheme 3), in which the electrophilic amidoacetone nitrile warhead is replaced by an unreactive trifluoroethylamino group, thus rendering it inactive against cathepsins K, B, L and S (Figure 5B). This result indicates that the accumulation of 6 is not due to binding to lysosomal cathepsins or other activated thiol-containing proteins. Furthermore, the fluorescence obtained with 6 colocalized with that of the lysosomal probe LysoTracker DND-99. Unfortunately, the nonbasic 7 does not have an appropriate intrinsic fluorescence, thus precluding its use as a control. However, further studies with a radiolabeled nonbasic cathepsin K inhibitor failed to show any sign of accumulation within cells, in contrast to the results obtained with a basic-nitrogen-containing analogue.^[36]

The pharmacokinetic properties of 4 and 6 are also consistent with lysosomotropic behaviour, as both have relatively high volumes of distribution. In rat-tissue-distribution studies, both 4 and 6 showed kidney, spleen, liver, and lung levels that were ~6–12-fold higher than those found in plasma. These organs have a high lysosome content, in contrast to fat and muscle, in which 4 and 6 levels were similar to those found in plasma. The nonbasic analogue 5 had similar tissue levels in both lysosome-rich and -poor organs and in plasma.^[36]

The results of the ABP whole-cell enzyme-occupancy assays for cathepsins B, L, and S described above, indicate that basic cathepsin K inhibitors have greater than anticipated activities against these off-target lysosomal/endosomal enzymes. This hypothesis was tested by using a functional cell-based assay of

cathepsin S activity. Cathepsin S is expressed in the acidic endosome of several antigen-presenting cell types where it plays an important role in antigen processing.^[5,39] Cathepsin S performs the final cleavage of a peptide known as the invariant chain (Ii), which occupies and blocks the peptide-binding groove of the MHCII complex. Cleavage of the Ii p10 fragment and its removal from this groove is required before the binding of antigenic peptide and trafficking of the complex for antigen presentation to CD4^+ T-cells can occur. The functional mouse cathepsin S cell-based assay employs an engineered B cell line (A20) that presents a specific ovalbumin-derived peptide via MHCII to a responsive T-cell hybridoma cell line (DO.11.10). Presentation of the ovalbumin peptide to the T-cell stimulates the latter to secrete interleukin-2, which is quantitated. A nonbasic, highly potent, and selective cathepsin S inhibitor (10, Scheme 3) showed similar potencies in mouse splenocyte enzyme-occupancy and enzyme assays, thus demonstrating that it is cell permeable. However, approximately two orders of potency were lost in the mouse antigen-presentation assay, possibly due to the requirement for a high degree of cathepsin S inhibition for functional activity (Table 4). In contrast, the basic inhibitors 4, 6, and 8, which are relatively

Table 4. Potencies of basic and nonbasic cathepsin S inhibitors against mouse splenocyte whole-cell enzyme-occupancy, whole-cell enzyme-occupancy, and functional antigen presentation assays			
Inhibitor	Inhibition of mouse cathepsin S IC_{50} (nM)		
	Enzyme	Cell (splenocyte)	Antigen presentation
10	13	40	1220
4	6350	480	6180
6	5400	318	6200
8	34100	740	8600
7	8500	6700	> 100000

weak against purified mouse cathepsin S (but which show increased activity in the splenocyte whole-cell enzyme-occupancy assay), exhibit activities in the antigen presentation assay that are of the same order as that of 10. These results suggest that the lysosomotropic properties of 4, 6, and 8 contribute to their potencies in the functional cell-based assay.^[36]

Of course, the lysosomotropic properties of basic cathepsin K inhibitors might also contribute to their functional efficacy, as the cathepsin K-mediated degradation of collagen occurs in the acidic lacunae beneath the osteoclasts, as well as in lysosomes within the osteoclasts.^[40] Indeed, increased basic inhibitor potencies in the cathepsin K HIG32 cell enzyme-occupancy assay was described above. Therefore, the increased potencies of basic compounds such as 4 and 6 against intracellular antitarget cathepsins B, L, and S may be compensated for by a corresponding increased potency against cellular cathepsin K activity. However, a comparison of the potencies of basic and nonbasic inhibitors against purified rabbit cathepsin K and the functional rabbit osteoclast bone-resorption assay does

not demonstrate any increased potency for the basic inhibitors (Table 5).^[44] Although the intrinsic potencies of the basic compounds 4 and 6 against rabbit cathepsin K are greater than

Table 5. Intrinsic potencies of basic and nonbasic cathepsin K inhibitors in purified rabbit cathepsin K enzyme assay and functional rabbit bone-resorption assay			
	Inhibition of cathepsin K, IC ₅₀ [nM]		Potency shift (fold)
	Rabbit cathepsin K	Rabbit bone resorption assay	
4	2.7	97	35
6	0.5	5	10
7	9.5	41	4
8	33	149	5
26	0.8	13	16

those of their nonbasic analogues 5 and 7 (due to a favourable electrostatic interaction with the S3 aspartic acid),^[29] the potency shifts between the purified-enzyme assay and the functional cell-based assay are roughly equivalent for both inhibitor types. Furthermore, examination of the potency shifts between purified rabbit cathepsin K and bone-resorption assays for 672 nonbasic and 196 P3 basic-nitrogen-containing amidoacetonitrile cathepsin K inhibitors (Figure 6), shows that both inhibitor types suffer approximately the same ~12–15-fold decrease in potency in the functional assay.

The lack of advantage of basic cathepsin K inhibitors in functional assays, coupled with the loss of selectivity in whole-cell assays has driven our search for a selective cathepsin K inhibitor towards nonbasic molecules. While this increases the challenge of achieving large selectivity ratios in enzyme assays, the approach seems more certain to provide functional selectivity in cell-based assays and might, by extension, provide a safer drug for clinical evaluation.

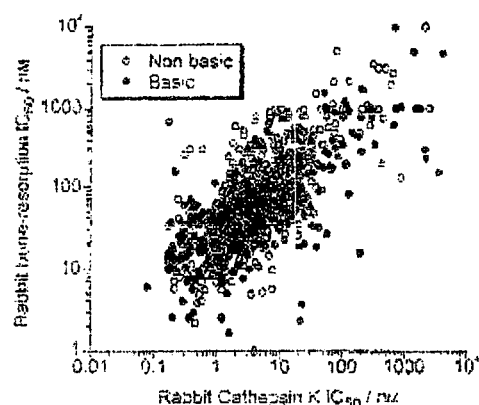
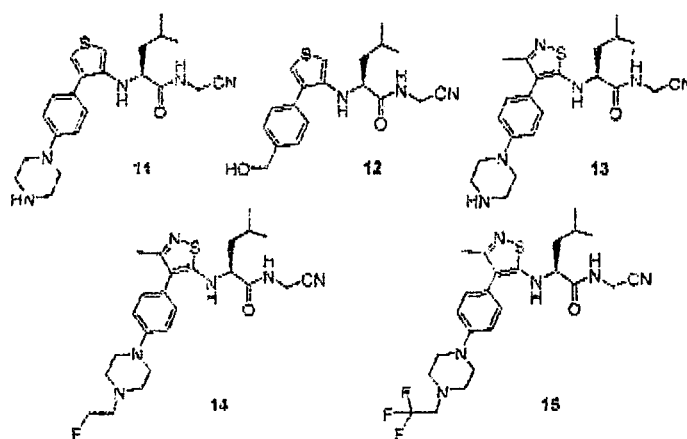


Figure 6. Relationship between rabbit cathepsin K enzyme and rabbit osteoclast bone-resorption assay potencies for basic and nonbasic α -amidoacetonitrile cathepsin K inhibitors. Basic cathepsin K inhibitors suffer on average a 12.2-fold shift (95% confidence limits, 8.6–15.5) between the enzyme and bone-resorption assay and nonbasic inhibitors suffer a 15.5-fold shift (95% confidence limits, 14.3–16.8; M. D. Percival, unpublished data).

Peptidomimetics and Nonbasic Inhibitors

Extensive SAR studies on the dipeptide nitrile series convinced us that it would not be possible to achieve an acceptable level of selectivity in the absence of a basic P3 group. In an attempt to identify a novel series with improved properties, we turned to peptidomimetics. X-ray crystallography of dipeptide inhibitors bound to cathepsin K shows that the P1–P2 amide forms hydrogen bonds to the protein with both the NH and carbonyl components. While replacement of this amide by a pyrimidine or purine has been demonstrated,^[18] the potency of the resulting inhibitors is dependent on the reactivity of the nitrile warhead and did not appear to us to be a viable series. Other P1–P2 amide replacements that we investigated led to a considerable loss of potency.

The P2–P3 amide bond appeared much more amenable to modification. It forms only one hydrogen bond to Gly66 with the NH, while the carbonyl projects into solution.^[41] This implies that the P2–P3 amide bond might be replaced with an H-bond-donating isostere without dramatic loss of potency. Previous experiments had shown that replacement of the entire amide bond with a phenyl ring resulted in compounds of modest potency and excellent pharmacokinetics (e.g., compound 8).^[42] Introduction of an aniline NH to form the important hydrogen bond to Gly66 led to a dramatic improvement in potency.^[43] The aniline nitrogen has low basicity and is not protonated in the biological milieu, thus making it an excellent H-bond donor. Replacing the aniline with an aminothiophene moiety provided a compound (11, Scheme 4) with optimal potency and selectivity. In this series, a basic P3 group provided compounds with excellent enzyme selectivity over off-target cathepsins. However, compounds with neutral P3 groups (e.g., 12) have poor selectivity over cathepsins L and S (Table 6). Additionally, the aminothiophene group had unacceptable levels of covalent binding in microsomal preparations, possibly due to amine oxidation (S. Day, unpublished results). Replacement of the aminothiophene with more electron-deficient heterocycles (e.g., 13) resulted in lower levels of covalent binding, but the selectivity problems with nonbasic P3 groups persisted.

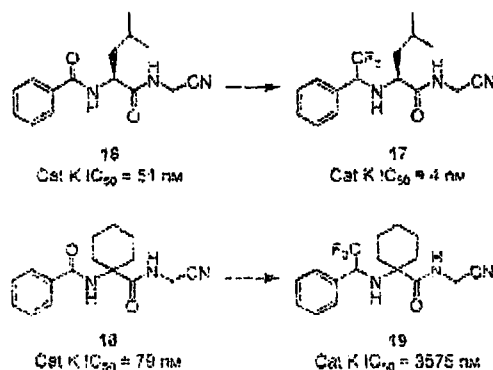


Scheme 4. Aminoheterocycle-based cathepsin K inhibitors.

Compd	Cat K	Enzyme IC ₅₀ (nM)		
		Cat B	Cat L	Cat S
6	0.2	896	2760	> 10000
8	7.8	3950	3725	2010
11	0.2	3465	122	40
12	0.3	703	103	104
13	1.7	2625	65	54
14	0.3	500	652	145
15	5.2	61	2169	6

As part of the effort to address this problem, we modified the basicity of the piperidine ring of compound 13 with fluoroalkyl substituents in an attempt to find a compromise between lysosomotropism and selectivity. A monofluoroethyl substituent led to a molecule (14) that was still basic but with a potency and selectivity profile that compare favourably to those of 13. In contrast, the trifluoroethyl analogue 15 appeared to be completely neutral, as judged by its nonpolar behaviour on silica-gel chromatography (calculated $pK_a \approx 1.4$). The potency and selectivity of this molecule were reduced considerably; this is consistent with other nonbasic molecules in this series, a result that prompted us to abandon attempts at pK_a modulation.

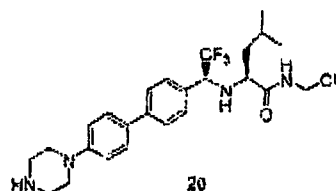
The observation that this β -CF₃ group rendered the nitrogen nonbasic was highly relevant to our parallel effort of P2–P3 amide-bond replacement. A version of this functional group with a secondary amine would seem perfectly suited to replacing the carbonyl group of the P2–P3 amide bond. This is indeed the case, as replacing this amide bond of the dipeptide inhibitor 16 with a trifluoroethylamine as a diastereomeric mixture results in compound 17, which has a tenfold increase in potency against cathepsin K (Scheme 5).^[44] It is noteworthy that this potency enhancement is only obtained in molecules with single P2 substituents, such as leucine. In the case of the P2 1,1'-cyclohexyl group (19) a significant loss in potency is obtained. This appears to be due to a steric interaction between the P2 alkyl group and the CF₃ moiety that prevents the



Scheme 5. Effect of CF₃ amide replacement on dipeptide series of cathepsin K inhibitors.

molecule from adopting a biologically active conformation. This trifluoroethylamine amide surrogate has previously been used to good effect by Zanda and co-workers.^[46]

Adding the potency-boosting P3 phenylpiperidine to this template provides a molecule (Scheme 6) with exceptional po-



Scheme 6. Trifluoroethylamine derivative 20, a 5 pM cathepsin K inhibitor.

tency and selectivity in enzyme assays. Compound 20 is a ~5 pM inhibitor of humanized rabbit cathepsin K and is > 10000-fold selective against other cathepsins.^[44] In this series of inhibitors, the active diastereomer is that with the S configuration at the CF₃ stereocentre. The R isomer is essentially inactive (data not shown). While the enhanced potency of the piperazine-containing 20 makes a nice showcase for the trifluoroethylamine amide replacement, its basic, lipophilic nature suggests that this compound will also be lysosomotropic. Data to support this are provided by potency in whole-cell assays, in which 20 is found to be three- to 64-fold more potent than in the corresponding enzyme assays (Table 3). Moreover, the extreme enzyme potency of this compound does not lead to enhanced functional activity. In the rabbit osteoclast bone-resorption assay,^[14] the potency of compound 20 is 12 nM, a 1200-fold shift over the cathepsin K IC₅₀. The reason for this is unclear, but might be due to the assay 'bottoming out' due to the high concentration of cathepsin K in the osteoclast. At face value though, these data do not support the hypothesis that the lysosomotropic properties of basic inhibitors would lead to enhanced cathepsin K inhibition in the acidic resorption lacunae of osteoclasts.

While nonbasic dipeptide inhibitors show poor potency and selectivity, the improved potency and selectivity of the trifluoroethylamine inhibitors prompted us to re-examine nonbasic P3 substituents as a means of avoiding the liabilities of lysosomotropism. Indeed, numerous aromatic P3 groups provided compounds with subnanomolar potency and high levels of selectivity against cathepsins B, L, and S. Pyridinyl (23), thiazolyl (24), and 4-substituted phenyl (25) all showed considerable promise (Table 7). With a wide range of molecules to choose from, preclinical pharmacokinetics and activity in the in vitro bone-resorption assay with rabbit osteoclasts were evaluated for a number of compounds. The added polarity of a sulfone (26) or sulfonamide (27) moiety in the 4-position of the bi-phenyl led to compounds with particularly attractive pharmacokinetics. On the basis of its overall profile, compound 26 (L-873724) was selected for further evaluation.^[46]

The lack of a basic group in 26 suggests that it should not have lysosomotropic properties. Indeed, the low volume of distribution (1.4 L kg⁻¹) observed in rat pharmacokinetics distin-

Table 2. Humanized rabbit cathepsin K inhibitors. Pharmacokinetics of compounds in ovariectomized rhesus monkeys.

Cmpd	R	Cat K ⁴⁰	Enzyme IC ₅₀ (nM)			Bone res. IC ₅₀ (nM)	% F	Rat pharmacokinetics	
			Cat B	Cat L	Cat S			V ₀ (L kg ⁻¹)	t _{1/2} (h)
21	Br	0.9	2992	13	54	111	—	—	—
22		0.2	1605	1473	303	44	9	3.5	3.1
23		0.3	2374	80	62	19	173	2.5	2
24		0.3	4818	876	240	52	145	4.4	4
25		0.6	5939	887	449	29	43	3.9	3
26		0.2	5239	264	178	13	40	1.4	3
27		0.2	1561	470	154	52	25	1.9	10

[a] Humanized rabbit Cat K⁴⁰.

guished it from basic cathepsin K inhibitors we have evaluated. When 26 was evaluated in whole-cell assays, the activity observed was comparable to that seen in purified-enzyme assays (Table 3). The shift between the rabbit enzyme potency and the functional osteoclasts bone-resorption assay was 16-fold, which is comparable to that observed for the lysosomotropic inhibitor 6 (Table 5).⁽⁴⁶⁾

While this nonbasic cathepsin K inhibitor showed comparable potency to the basic inhibitor 6 in the osteoclast bone-resorption assay, it was unclear whether a nonbasic inhibitor would have comparable activity *in vivo*. Since the resorption lacuna of an osteoclast is acidic, it would seem reasonable to assume that a basic derivative would concentrate in the regions of high cathepsin K activity, leading to efficacy at lower systemic exposure. Potential differences in compound distribution and the question of whether the *in vitro* bone resorption could accurately distinguish potent inhibitors contributed to this uncertainty. The concept was tested in an ovariectomized rhesus monkey model wherein N-telopeptide fragments (NTx) resulting from collagen degradation were quantified as a measure of bone resorption. Previously, the basic inhibitor 6 (L-006235) had given a 58% reduction in urinary NTx over six days of oral dosing at 3 mg kg⁻¹ (Figure 7A).^(26,47) The nonbasic inhibitor 26 (L-873724), was tested in this model at the equivalent 3 mg kg⁻¹ dose and gave an identical 68% inhibition of uNTx (Figure 7B). Notably, the uNTx levels return to baseline three days after cessation of treatment; this indicates the reversibility of this mechanism and distinguishes it from bisphosphonate treatment.⁽⁴⁷⁾ Clearly, basicity is not required for efficacy in cathepsin K inhibitors.

An X-ray crystal structure of sulfonamide 27 bound to cathepsin K has been obtained at 2 Å resolution (Figure 8). The cysteine adducts (thioimide), P1–P2 amide bond, and P2 leucine moiety all bind in analogous fashion to the dipeptide inhibitors. The CF₃ group is directed out of the active site towards water, and the NH of the trifluoroethylamine forms a well-defined hydrogen bond to the carbonyl of Gly66 that is similar to the hydrogen bond formed by the P2–P3 amide of the dipeptide inhibitors. However, the sp² hybridization of this nitrogen allows a superior orientation of this hydrogen bond relative to the sp³ nitrogen of an amide. The aromatic ring is positioned over the hydrophobic glycine shelf formed by the π -surface of Gly65 and Gly66. While a similar interaction is found in the dipeptide inhibitors, the sp³ benzylic carbon allows for an optimal orientation of this aromatic ring. The distal phenyl ring occupies the S3 binding site, while the sulfonamide group projects into water and has no significant interaction with the enzyme. This is consistent with the forgiving SAR of this portion of the inhibitor.

The fact that the CF₃ group itself does not have any binding interactions with the enzyme suggests that it may be replaced by other electron-withdrawing groups without impacting potency. This concept was explored in a brief SAR study (Table 8). Compounds containing replacements for the CF₃ group were evaluated for amine basicity as well as for potency against cathepsin K. To estimate basicity, a simple TLC method in the presence of acetic acid was used that correlated reasonably well with calculated pK_a (Table 8). Both a pentafluoroethyl group (28) and a phenylsulfone (29) in this position provided compounds with similar activity to that of the CF₃ derivative.

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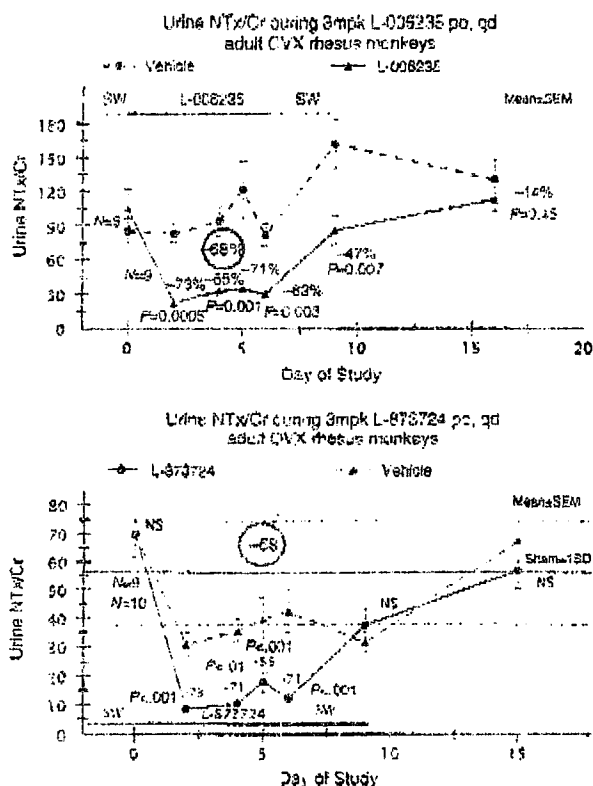


Figure 7. Efficacy of A) 0.6 mg kg⁻¹ L-006235 and B) 1 mg kg⁻¹ L-873724 in the ovariectomized rhesus monkey model.

Table 1. Comparison of inhibitors with different replacements

Compound	R	IC ₅₀ [nM] Cat K ^[a]	R ₁ ^[b]	pK _a ^[c]
27	CF ₃	0.9	0.40	1.3
28	CF ₂ CF ₃	2.4	0.43	1.9
29	4-MeSO ₂ Ph	2.5	0.38	4.6
30	CH ₃	988	0.03	6.7
31	CN	30	0.67	0.7

[a] Humanized rabbit enzyme. [b] TLC on SiO₂ plates with 40% EtOAc/i% HOAc/hexanes. [c] Calculated by using ACD/PC.

In contrast, a methyl substituent leads to an inactive compound (30), probably due to the basic nature of the resulting amine. Surprisingly, a nitrile substituent (31), while generating a nonbasic amine, resulted in a marked loss in potency; this suggests that the CF₃ group does more than simply neutralize the amine. We propose that the CF₃ group or a similar large substituent imposes a steric constraint on the orientation of the aromatic ring, forcing it to adopt a biologically active conformation. The much smaller nitrile results in a low barrier to

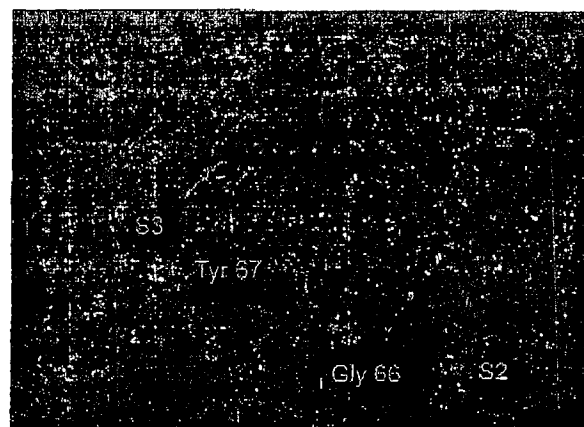


Figure 8. X-ray crystal structure of compound 27 bound to cathepsin K showing the P2-P3 trifluoroethylamine group and the hydrogen bond it makes to Gly66 (PDB ID: 2FDZ). Reprinted from ref. [46], Copyright (2006), with permission from Elsevier

rotation for the aromatic ring and is unable to enforce this preferred conformer.

Conclusion

Lysosomotropism is a common feature of many marketed drugs (e.g., fluoxetine, chlorpromazine, imipramine, and azithromycin). This physical property might in fact lead to improved pharmacokinetic properties: a drug that is sequestered in the lysosomes will have reduced exposure to hepatic CYPs, thus allowing the lysosome to act as a depot that will release the drug slowly and result in a long terminal half-life. One can also imagine a scenario in which the target protein is located in the lysosome. In this case, a lysosomotropic compound might provide pharmacological activity with low systemic exposure. This could be the case with cathepsin S, for which an augmentation of functional cellular activity by basic inhibitors was observed. Additionally, when all off-target proteins are located in the same acidic compartment, lysosomotropism would not result in a change in functional selectivity. Cysteine cathepsins can also be active as secreted and cell membrane-associated enzymes. In these cases, no effect of lysosomotropism on inhibitor potency may be expected. However, in the case of cathepsin K inhibitors, lysosomotropic compounds have no advantage in potency over nonbasic analogues in either the functional cell-based bone-resorption assay or in a primate model of osteoporosis. This suggests that activity against off-target lysosomal cathepsins will be increased by a basic lysosomotropic compound in the face of unaltered cathepsin K activity, potentially leading to undesired pharmacological effects. Therefore, in order to preserve the selectivity of a cathepsin K inhibitor, it is prudent to design nonbasic inhibitors. Nonbasic inhibitors of cathepsin K in which a trifluoroethylamine has replaced the P2 amide bond have subnanomolar potency and show high selectivity over other cathepsins.

Compound 26 (L-873724) has good pharmacokinetic properties and is active in a monkey model of osteoporosis.

Acknowledgements

We thank J.-P. Falgueyret, R. Oballa, S. Day and T. Le Riche for the opportunity to present their unpublished data and we thank V. Devanarayan for statistical analyses.



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Received: April 7, 2006

Published online on August 21, 2006

The discovery of odanacatib (MK-0822), a selective inhibitor of cathepsin K

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Received 8 November 2007; revised 17 December 2007; accepted 19 December 2007

Available online 15 January 2008

Dedicated to the memory of Dr. Gideon Rodan, deceased, January 1, 2006

Abstract—Odanacatib is a potent, selective, and neutral cathepsin K inhibitor which was developed to address the metabolic liabilities of the Cat K inhibitor L-873724. Substituting P1 and modifying the P2 side chain led to a metabolically robust inhibitor with a long half-life in preclinical species. Odanacatib was more selective in whole cell assays than the published Cat K inhibitors balicatib and relacatib. Evaluation in dermal fibroblast culture showed minimal intracellular collagen accumulation relative to less selective Cat K inhibitors.

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Cathepsin K (Cat K) is a lysosomal cysteine protease that is highly expressed in osteoclasts, the cells responsible for bone degradation during bone remodeling. Type I collagen is a major component of bone and Cat K has high collagenase activity, particularly at the acidic pH that is required to dissolve the calcium hydroxyapatite component of bone. Emerging evidence that Cat K is the primary enzyme involved in osteoclastic bone resorption has made it an important target for the treatment of osteoporosis.¹ Several studies have shown that Cat K deficiency leads to an increase in bone mineral density (BMD).² Pharmacological studies of Cat K inhibitors in rats³ and monkeys⁴ have shown reductions in biochemical markers of bone resorption and

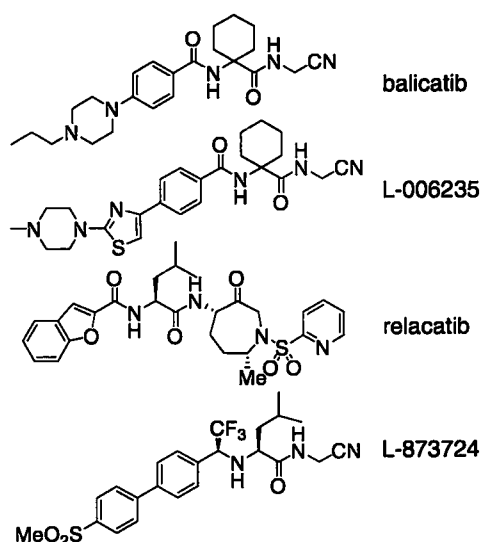


Figure 1. Published Cat K inhibitors.

Keywords: Cathepsin K; Cysteine protease; Odanacatib; MK-0822; Osteoporosis.

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increased BMD. Recently, clinical data have been disclosed for the Cat K inhibitor balicatib (Fig. 1), demonstrating a reduction of biochemical markers of bone resorption and increases in BMD over 1 year of treatment.⁵

We have previously reported on our effort to identify potent and selective inhibitors of Cat K.⁶ Our initial proof-of-concept compound, L-006235, was highly selective over cathepsins B, L, and S in enzyme assays and had good pharmacokinetics.⁷ However more detailed studies revealed that the selectivity profile was severely eroded in more physiologically relevant cell-based enzyme occupancy assays.⁸ The relevance of these data was demonstrated in a Cat S-dependent B cell line assay in which L-006235 blocked antigen presentation. This loss of selectivity in cell-based assays can be explained by the lysosomotropic properties of these basic, lipophilic compounds. Since off-target cathepsins are found in lysosomes (pH 4–5), the lysosomal accumulation of a basic Cat K inhibitor results in an apparent increase in potency on these anti-targets. High selectivity will be important to the success of a development candidate, precluding the use of a basic moiety in inhibitor design.

Balicatib is structurally related to L-006235 and is also lysosomotropic.⁸ Whole cell assays showed poor selectivity as was observed for L-006235. Recently it was announced that the Phase II development of balicatib has been discontinued due to skin rash and rarer incidences of morphea-like skin changes.⁹ Another Cat K inhibitor in clinical development, relacatib, is non-basic and therefore not lysosomotropic, but has poor selectivity over cathepsins B, L, S, and V in enzyme assays.¹⁰

Our initial attempts to remove the basic substituent in P3 led to Cat K inhibitors with poor activity, selectivity, and pharmacokinetic properties. The loss in activity was attributed to the loss of a beneficial ionic interaction between the charged amine and Asp⁶¹ in the S3 pocket.⁷ Since this residue is absent in the off-target cathepsins, the potencies against the anti-targets were not dramatically affected and selectivity was consequently reduced. Replacing the P2 amide bond with a trifluoroethylamine provided a 10- to 20-fold increase in potency on Cat K.¹¹ Using this motif, neutral inhibitors with high potency and selectivity were prepared. L-873724 was identified as a 0.2 nM Cat K inhibitor with >800-fold selectivity over other cathepsins. In vivo studies showed that this neutral Cat K inhibitor suppressed biochemical markers of bone resorption in a rhesus monkey model.¹²

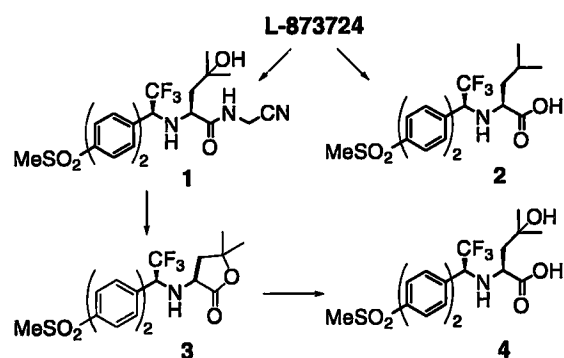
L-873724 has metabolic liabilities that prevented its further development. An analysis of its metabolic profile provided guidance for addressing these liabilities. Subsequent blocking of the key metabolic sites resulted in the identification of odanacatib (MK-0822) which is currently in clinical development.

The pharmacokinetics of L-873724 in rat, dog, and monkey have been reported.¹² The short half-life (2 h) and clearance (Cl = 7.5 mL/min/kg) in monkey raised

concerns that this compound may not be suitable for once-daily dosing in humans. Incubations in human hepatocytes showed that the major route of metabolism was hydroxylation on the methine of the leucine side chain (1)¹³ as shown in Scheme 1. A minor pathway leading to the hydrolysis of the P1 amide bond (2) was also observed. Analysis of plasma from several species showed several circulating metabolites. In particular, the lactone 3 was found to be circulating at high levels in rhesus monkey (10× L-873724 concentration at 8 h) even though it was only a minor component in rat plasma. To understand the potential relevance of this metabolite to humans, a synthetic standard of 3 was incubated in fresh plasma from rat, rhesus monkey, and human. In all three species, the lactone hydrolyzed to hydroxyacid 4 over the course of the incubation (37 °C, 1 h). However this hydrolysis was much more efficient in rat plasma (50% conversion) than in rhesus monkey plasma (5% conversion). The extent of hydrolysis in human plasma was intermediate (28–40% conversion) leading us to conclude that this metabolite would likely circulate in humans.

Further investigations revealed that the leucine hydroxylation occurred exclusively due to CYP3A activity. Adding the CYP3A inhibitor ketoconazole to the hepatocyte incubations resulted in a near-complete blockade of metabolism. When L-873724 was incubated for 1 h in pooled human microsomes, only a 10% recovery of the parent drug was observed. Microsomal incubation in the presence of a CYP3A inhibitory antibody gave >90% recovery of parent drug. Finally, incubation with several recombinant CYPs (1A1, 2D6*1, 3A4, 2C9*1, 2C9*2, 2C9*3, and 2C19) showed that only CYP3A led to detectable metabolite formation. Metabolism by only CYP3A can lead to variable exposure when co-dosed with other drugs that are CYP3A inhibitors and inducers.

To address the metabolic liabilities listed above, both the P1 and P2 residues were modified to minimize leucine hydroxylation, amide hydrolysis, and lactonization (Table 1). Most P1 substituents resulted in a loss of activity. One of the more active compounds (14) was provided by incorporation of an (*S*)-benzyl group, but the corresponding increase in potency on cathepsins B, L, and S resulted in a poor selectivity profile. The (*R*)-



Scheme 1. Metabolic pathways for L-873724 based on in vitro and in vivo studies.

Table 1. In vitro activity of L-873724 analogues with P1 substitution and stabilized P2 groups

Compound	R ¹	R ²	IC ₅₀ ^a (nM)			
			Cat K ^b	Cat B	Cat L	Cat S
L-873724	<i>i</i> -Bu	H, H	0.2	5239	264	178
12	<i>i</i> -Bu	(<i>S</i>)-Me, H	1.2	>10,000	3389	2237
13	<i>i</i> -Bu	(<i>R/S</i>)-Ph, H	0.6	3453	667	217
14	<i>i</i> -Bu	(<i>S</i>)-Bn, H	0.6	953	67	38
15	<i>i</i> -Bu	(<i>R</i>)-Bn, H	5.1	>10,000	2940	2834
16	<i>i</i> -Bu	1,1-Me ₂	4.0	>10,000	>10,000	2489
17	<i>i</i> -Bu	1,1- <i>c</i> Pr	0.3	>10,000	456	266
18	<i>i</i> -Bu	1,1- <i>c</i> Bu	16	>10,000	>10,000	>10,000
19		1,1- <i>c</i> Pr	0.3	>10,000	262	56
20		1,1- <i>c</i> Pr	0.4	>10,000	2650	170
21		1,1- <i>c</i> Pr	0.2	100	472	17
22		1,1- <i>c</i> Pr	<0.2	1528	110	15
23		1,1- <i>c</i> Pr	1.5	434	750	89
24 (odanacatib)		1,1- <i>c</i> Pr	0.2	1034	2995	60

^a IC₅₀ values represent an average of at least three titrations. Standard deviations for these assays were typically within 35% of the IC₅₀ values.^b Humanized rabbit Cat K (Ref. 6); others are human enzymes. See Ref. 17 for assay conditions.

benzyl group provided a compound (**15**) that is 8-fold less potent, suggesting that there is limited space for substituents in the back of the P1 pocket. The gem-dimethyl substituted **16** shows similar behavior. The optimal substituent was a 1,1-cyclopropane ring (**17**) which gave an in vitro profile similar to that of the unsubstituted aminonitrile. Increasing the ring size to a 1,1-cyclobutane substituent (**18**) resulted in a 50-fold loss in potency, again demonstrating the restrictions of the P1 pocket.

Previous SAR studies of cathepsin inhibitors have shown that the P2 substituent is critical to achieving both potency and selectivity.⁷ For Cat K, an isobutyl substituent in P2 has been found to provide an optimal in vitro profile. The metabolic liability of this group led us to attempt metabolic stabilization by introducing halogens in various locations. Even though the steric

differences of the resulting inhibitors are subtle, the impact on both Cat K potency and selectivity is significant (Table 1). For example, the two diastereomers **19** and **20**, while having similar potency on Cat K, show a 10-fold difference in Cat L potency. The difluoropropyl side chain **21** has similar potency on Cat K, but has poor selectivity on Cat B, L, and S. The dichloroethyl side chain **22** has increased potency against all the tested cathepsins, while the trifluoroethyl derivative **23** loses activity on Cat K, but gains activity on Cat B. The optimal combination of potency and selectivity was found with the 4-fluoroleucine derivative **24** (odanacatib, MK-0822). It was particularly gratifying to note that this compound had little activity on Cat L, since the Cat L^{−/−} mouse shows several undesirable phenotypes.¹⁴ Evaluation against other cathepsins gave IC₅₀s of 795 nM versus Cat F and 762 nM versus Cat V. There

was no measurable activity versus cathepsins C, H, and Z ($>10 \mu\text{M}$). Odanacatib is a reversible inhibitor of Cat K with on- and off-rates of $5.3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ and 0.0008 s^{-1} ($t_{1/2} \sim 14 \text{ min}$), respectively.

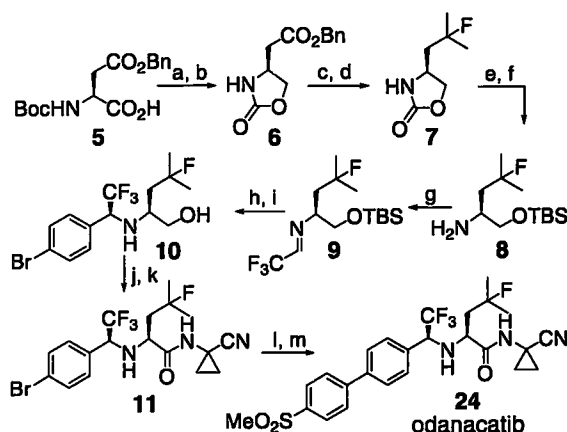
The inhibitors described above were prepared according to methods analogous to those published previously.¹² The route is exemplified by the synthesis of odanacatib (24) in Scheme 2. The synthesis of 4-fluoroleucinol begins with commercially available aspartic acid derivative 5. Reduction of the acid followed by activation with tosylic anhydride provides the cyclic carbamate 6. Methyl Grignard addition to the benzyl ester and fluorination of the resulting tertiary alcohol gave fluoride 7. Carbamate hydrolysis with barium hydroxide proceeded in quantitative yield and the resulting amino alcohol was silylated to facilitate isolation and provide stereochemical control in the subsequent imine addition step. Condensation of 8 with trifluoroacetaldehyde hemiacetal provided imine 9 which was treated with bromophenyllithium to give the trifluoroethylamine in a 10:1 ratio of *S,S* to *R,S* diastereomers. Desilylation followed by periodic acid oxidation¹⁵ under anhydrous conditions gave the carboxylic acid which was crystallized as the dicyclohexylamine salt to give $>99\%$ de. Amide formation with cyclopropanecarboxamide proceeded using HATU to provide 11. Suzuki coupling followed by sulfide oxidation then provided odanacatib 24. The P2 amino acids and aminoalcohols required for analogues 19–23 were prepared according to literature methods and converted into final products as described in Scheme 2. Details are provided in Supplementary material. Additional synthetic routes to this class of compounds have been reported.¹⁶

The potency of odanacatib was evaluated in a functional bone resorption assay using rabbit osteoclasts cultured on bovine bone. Since the majority of our Cat K inhib-

itors are less potent on rabbit Cat K than on human Cat K, a 'corrected bone res' value has been calculated based on the difference between the rabbit and human potencies (Table 2). This allows comparisons of functional potency to be made between compounds in a human context. Odanacatib showed potency similar to L-873724 and was 3- to 4-fold more potent than balicatib and relacatib. The reduced potency of inhibitors in this assay relative to the Cat K enzyme assay may reflect the high fractional inhibition of Cat K required to inhibit bone resorption in osteoclasts.

Odanacatib was evaluated in whole cell enzyme occupancy assays using human HepG2 cells (Cat B, L) and Ramos cells (Cat S) for selectivity versus off-target cathepsins (Table 3) as previously described.¹⁷ In these whole cell assays, odanacatib showed similar potencies to those obtained in the corresponding purified enzyme assays. This provided a high degree of selectivity versus the corrected bone res Cat K assay, particularly compared to the other catibs. The lowest selectivity of odanacatib is against Cat S, so its potential to inhibit Cat S in vitro was addressed with two functional assays. Odanacatib was a weak inhibitor of antigen presentation, measured in a mouse B cell line ($\text{IC}_{50} = 1.5 \pm 0.4 \mu\text{M}$), compared to the Cat S inhibitor LHVS ($\text{IC}_{50} = 0.001 \mu\text{M}$) in the same assay.⁸ Odanacatib also showed weak inhibition of the processing of the MHC II invariant chain protein lip10 in mouse splenocytes compared to LHVS (minimum inhibitory concentration 1–10 μM versus 0.01 μM , respectively).¹⁸ The potency of odanacatib is 4-fold greater against isolated mouse Cat S than human Cat S, therefore the potential for Cat S mediated immunosuppression appears to be low.

Cathepsins B, K, L, S, and V are all expressed in skin tissue and have some ability to degrade collagen and



Scheme 2. Reagents and conditions: (a) $\text{ClCOO}i\text{Bu}$, NMM, NaBH_4 , DME, 85%; (b) Ts_2O , pyr, dichloroethane, 83%; (c) MeMgBr , toluene/THF, 85%; (d) DAST, CH_2Cl_2 , 60%; (e) $\text{Ba}(\text{OH})_2$, $\text{EtOH}/\text{H}_2\text{O}$, 100%; (f) TBSCl , Et_3N ; (g) $\text{CF}_3\text{C}(\text{OH})\text{OEt}$, PhH, 88% (two steps); (h) BrPhLi , THF; (i) TBAF, THF, 75% (two steps); (j) H_5IO_6 , CrO_3 , CH_3CN , 60%; (k) 1-amino-1-cyanocyclopropane hydrochloride, $i\text{-Pr}_2\text{NEt}$, HATU, DMF, 80%; (l) $\text{MeSPhB}(\text{OH})_2$, PdCl_2dppf , Na_2CO_3 , DMF, 70%; (m) H_2O_2 , $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, Bu_4NHSO_4 , EtOAc , 97%.

Table 2. Cat K enzyme activity and functional bone resorption assay

Compound	IC_{50} (nM)			
	Human Cat K	Rabbit Cat K	Rabbit Bone Res ^a	'Corrected' Bone Res ^c
L-873724	0.2	0.8	13 ± 1	3
Odanacatib	0.2	1.0	23 ± 6	5
Balicatib	0.6	2.7	97 ± 13	22
Relacatib	0.5 ^b	0.5	15 ± 2	15

^a IC_{50} values represent an average of at least three titrations $\pm$ SEM.

^b Humanized rabbit Cat K.

^c Bone res $\text{IC}_{50} \times \text{hCat K } \text{IC}_{50} / \text{rabCat K } \text{IC}_{50}$.

Table 3. Whole cell potency on cathepsins K, B, L, and S

Compound	Cat K 'Corrected' Bone Res	IC_{50} (nM)		
		Enzyme occupancy		
		Cat B (HepG2)	Cat L (HepG2)	Cat S (Ramos)
L-873724	3	4807	1221	95
Odanacatib	5	1050	4843	45
Balicatib	22	61	48	2900
Relacatib	15	14	2	8

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extracellular matrix. A key pathway for collagen degradation by fibroblasts involves MMP-dependent partial digestion and phagocytosis, followed by cysteine cathepsin-dependent intracellular degradation.^{19,20} We hypothesized that the fibrotic events caused by balicatib⁹ are due to inhibition of multiple cathepsins by virtue of its lysosomotropic nature, resulting in a build up of matrix. A cellular model was used in which primary human dermal fibroblasts (HDF) were cultured in 3-D collagen gel and inhibitors added for the final 3 days.²¹ The cells were removed from the collagen by collagenase treatment, fixed and permeabilized, and intracellular type I collagen detected using an anti-type I collagen mAb and a FITC-labeled 2°Ab with quantitation by flow cytometry (see Supplementary material). Balicatib and the non-selective cathepsin inhibitors relacatib (Fig. 2) and E64/E64d (data not shown) caused a 3- to 7-fold accumulation of intracellular Type I collagen in HDF at concentrations of 1–10 μ M. In contrast, an inactive structural analogue of balicatib (compound 2 in Ref. 8) and an inactive diastereomer of relacatib (compound 13 in Ref. 10) showed less than 1.5-fold accumulation of collagen at concentrations up to 10 μ M (data not shown). The high degree of cellular selectivity of odanacatib was again demonstrated by its minimal effect on collagen accumulation, even at the relatively high concentration of 10 μ M (Fig. 2).

Odanacatib exhibited excellent metabolic stability in hepatocyte incubations across several species. In standard incubations (2×10^6 cells/mL, 20 μ M, 2 h), a 96% recovery of parent was found in rat hepatocytes and a 98% recovery was obtained in rhesus monkey hepatocytes. High recovery (>99%) was observed in both dog and human hepatocyte incubations.

The pharmacokinetics of odanacatib were evaluated in several preclinical species (Table 4). This molecule is highly crystalline with low aqueous solubility, and oral

Table 4. Pharmacokinetics of odanacatib (0.5% methocel + 0.2% SDS as oral vehicle)

Species	po Dose (mg/kg)	%F	Cl (mL/min/kg)	Half-life (h)	Vdss (L/kg)
Rat	10	8	2.0	6	1.1
Dog	5	6	0.13	57	0.6
Rh. Monk.	5	7	6.1	18	1.6

bioavailability was found to be highly dependent on vehicle, dosage, and sample preparation. For example, in the dog, the oral bioavailability when dosed as a suspension in 0.5% methocel was only 6% at 5 mg/kg, but increased to 100% when dosed as an amorphous dispersion prepared by adding a PEG-200 solution of the compound to methocel with sonication. Similarly, the bioavailability in rats when dosed at 5 mg/kg as a solution in PEG-400 was found to be 38%. The half-lives were long in all species and were consistent with the observed metabolic stability.

In conclusion, by blocking the sites of metabolism on L-873724, a compound was identified with minimal in vitro metabolism and long half-lives in preclinical species. Odanacatib has greater bone resorption activity and greater selectivity versus off-target cathepsins than do balicatib and relacatib. This reduced activity against other cathepsins may be responsible for the lower level of undesired collagen accumulation in skin fibroblasts compared to balicatib and relacatib. Odanacatib is currently in clinical development for the treatment of postmenopausal osteoporosis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.047.

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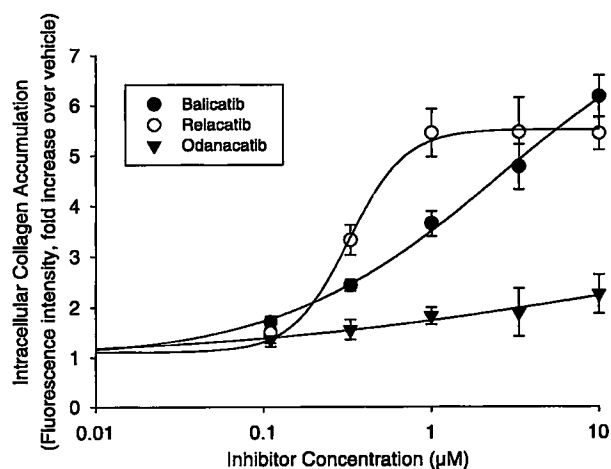


Figure 2. Effect of balicatib, relacatib, and odanacatib on the intracellular accumulation of Type I collagen in cultured primary human dermal fibroblasts. The data represent the average $\pm$ SD for at least $n = 2$ experiments for each inhibitor. A similar profile was observed using cells from a second donor.

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Cathepsin K Inhibitors: A Novel Target for Osteoporosis Therapy

SA Stoch¹ and JA Wagner¹

Osteoporosis is characterized by low bone mass with skeletal fragility and an increased risk of fracture. This bone loss is brought about by an imbalance between bone resorption and formation. Cathepsin K is the most abundant cysteine protease expressed in the osteoclast and is believed to be instrumental in bone matrix degradation necessary for bone resorption. Cathepsin K inhibitors represent a novel target for developing agents to treat osteoporosis and other disorders characterized by increased bone resorption.

In the United States, ~10 million Americans have osteoporosis (8 million women and 2 million men), while another 34 million individuals have osteopenia, resulting in a significant proportion of the population to be deemed "at risk." Osteoporosis is most commonly found in postmenopausal women. Men account for 20% of patients with osteoporosis. The major current anti-osteoporotic therapies include treatment using bisphosphonates (alendronate, risedronate, ibandronate, and zoledronate), estrogens, selective estrogen receptor modulators (raloxifene, bazedoxifene), and parathyroid hormone (PTH). Other niche treatments include calcitonin, vitamin D derivatives, and strontium (in some countries). Except for PTH and strontium, these drugs inhibit bone resorption. In the next 5 years, additional therapies are likely to be approved, including the anti-RANK-ligand monoclonal antibody (denosumab), and cathepsin K inhibitors.

Osteoclastic bone resorption requires two processes: demineralization of the inorganic bone components and degradation of the organic bone matrix (Figure 1). These two processes occur in the osteoclast in a coordinated fashion by two separate mechanisms. The first phase involves acid secretion by the osteoclast into the resorption lacunae, and the second phase is the organic matrix degradation by cysteine proteases. An acidic microenvironment is required for bone resorption, both to dissolve the mineral component of bone and to aid protein matrix digestion. This unique metabolic milieu is achieved by lowering the pH in the resorption lacunae via acid secretion by the osteoclast.

Cathepsin K is the most abundant cysteine protease expressed in the osteoclast and is believed to be instrumental in bone

matrix degradation necessary for bone resorption. Cathepsins have known collagenolytic activity under acidic conditions and cathepsin K is capable of degrading type-1 collagen, the major component of bone matrix.¹ The finding of cathepsin K deficiency in pycnodysostosis,² an osteopetrotic disorder characterized by decreased bone resorption, further underscores the importance of this enzyme as a potential target for developing agents to treat osteoporosis and other disorders characterized by increased bone resorption.

CHEMISTRY AND ACTIONS OF CATHEPSIN K

Cathepsin K is a member of the papain family of cysteine proteases and is both selectively and highly expressed in osteoclasts that mediate bone resorption.³ Activated cathepsin K is capable of degrading several components of bone matrix, including type I collagen (which accounts for ~90% of bone matrix), osteopontin, and osteonectin.

Cathepsin K was originally cloned from rabbit osteoclasts⁴ and thereafter from human osteoclasts and shown to be homologous to other cathepsins.⁵ Bone resorption is mediated by osteoclasts, which attach to the bone surface, thereby generating an acidified subosteoclastic bone-resorbing compartment.⁶ While cathepsin K is highly expressed in osteoclasts, very low levels exist in other tissues, including the heart, liver, and lung.⁷ The enzyme can be detected extracellularly in bone resorption lacunae and intracellularly in vesicles, granules, and vacuoles throughout the cytoplasm of osteoclasts.⁸

DESIGN OF INHIBITORS

The designing of specific cathepsin K inhibitors has progressed over recent years. The initial cathepsin K inhibitors bound irreversibly to the cysteine protease, thereby conferring the theoretical possibility of generating antigenic and immunologic complications, if used chronically. Subsequent inhibitors bound reversibly, but lacked adequate selectivity. Recently, more potent reversible inhibitors of cathepsin K have been generated that contain less-reactive functional groups, which is a desirable property for inhibitors intended for chronic use.⁹ While many

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Received 26 September 2007; accepted 16 October 2007; advance online publication 12 December 2007. doi:10.1038/sj.cpt.6100450

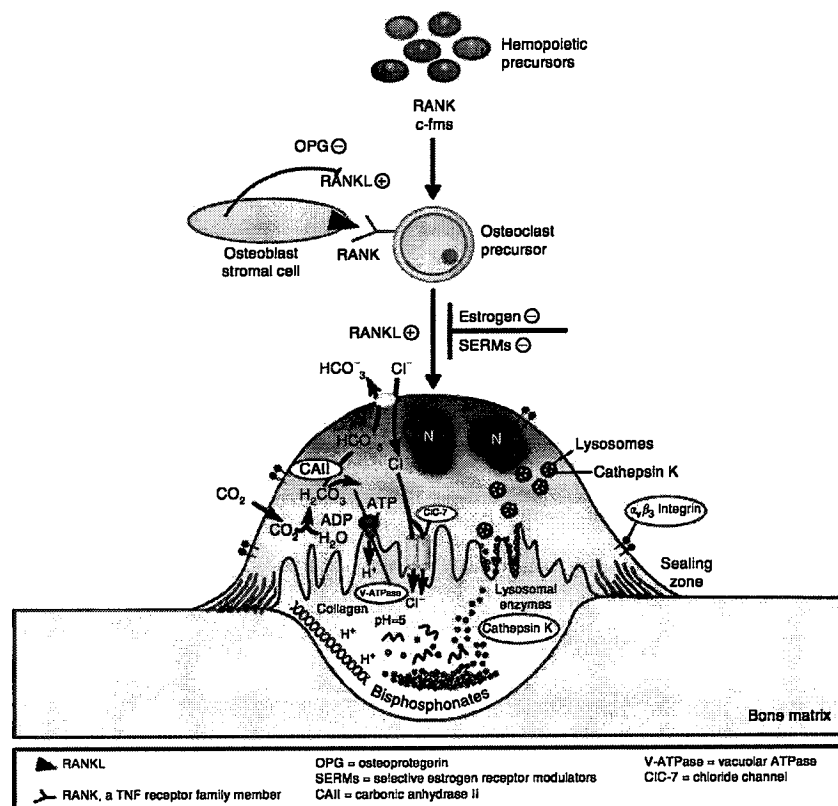


Figure 1 Diagram highlighting potential therapeutic targets, including cathepsin K, for pharmacological manipulation.

of these approaches to cathepsin K inhibition involved peptide molecules, potency and selectivity were hampered by their high molecular weights and poor pharmacokinetic properties. However, newer classes of lower molecular-weight compounds with a high degree of selectivity for inhibition of cathepsin K have now been identified.¹⁰

IN VITRO STUDIES

As part of profiling preclinical candidates, a number of *in vitro* studies testing cathepsin K inhibitors have been performed. Several peptide inhibitors of cathepsin K demonstrate potent inhibition of osteoclast-mediated bone resorption in a concentration-dependent manner using the PTH-stimulated fetal rat long bone model.¹¹ Given the challenges of culturing human osteoclasts from bone marrow cultures,¹² human osteoclastoma-derived osteoclasts which are phenotypically identical to human osteoclasts have been used for *in vitro* testing of novel antiresorptive compounds.¹³ Importantly, the potency of cathepsin K inhibitors using these cells correlates with the results obtained in assays based on recombinant human cathepsin K ($r^2 = 0.80$).¹³

IN VIVO STUDIES

A number of animal models, including rodents, rabbits, and non-human primates, have been used to evaluate potential osteoporosis therapies before testing in humans. Monkey cathepsin K is identical to mature human cathepsin K both in sequence and kinetics.¹⁴ The similarities between the human

and monkey cathepsin Ks, coupled with the knowledge that the ovariectomized (OVX) monkey is an excellent model for human osteoporosis,¹⁵ indicate that these models will be appropriate for evaluating inhibitors *in vivo*.

In addition to using preclinical animal models to define efficacy and pharmacokinetic and pharmacodynamic correlations to guide initial dosing targets for the clinic, animal models are also required from a regulatory standpoint. US and EU guidances for the development of anti-osteoporosis agents require preclinical demonstration of bone of normal quality (architecture, mass, and strength) in two species (rodent and non-rodent; ovariectomized). It is generally accepted that the ovariectomized rat is a useful model of human bone loss because it responds similarly to humans in regard to effects of mechanical forces as well as to hormone and drug treatment. However, there is less unanimity regarding the relevance of mouse models of postmenopausal bone loss. Although mice are readily available and the knockout and transgenic mouse models lend themselves to elegant experimentation and genetic analysis, the mouse strains differ in peak bone mass and susceptibility to bone loss.¹⁶ Furthermore, differences exist in how the human and mouse skeletons respond to estrogen.¹⁶ Nevertheless, the mouse model has utility in evaluating new chemical entities and their effects on mitigating bone loss.

Mouse models

Knockout mouse. The cathepsin K knockout mouse serves as an excellent model for analyzing the two distinct effects of

osteoclast-mediated bone resorption: demineralization and organic matrix degradation,¹⁶ and closely captures the phenotype of the human disease pycnodysostosis. Cathepsin K-deficient mice, generated by targeted disruption of the cathepsin K gene exhibit an osteopetrotic phenotype characterized by thick bone trabeculae.¹⁷ There is evidence confirming that these findings are associated with increases in bone strength.¹⁸

Ovariectomized mice. Cathepsin K inhibitors are usually designed to demonstrate activity against the human enzyme, and are significantly less potent (in the order of two orders of magnitude) against mouse and rat cathepsin K.²⁰ Nevertheless, cathepsin K inhibitors have been shown to prevent bone loss in OVX mice²¹ without appearing to disturb the anabolic action of PTH, suggesting that the combination of a cathepsin K inhibitor with an anabolic agent may offer additional bone mass increases when compared with that seen with a bisphosphonate and an anabolic. The predictive nature of this finding will need to be confirmed both in additional clinical models and in the clinic.

Rat models

Although the OVX rat model is a classic animal model for evaluating bone active compounds, the rat is not the preferred species for testing cathepsin K inhibitors because the cathepsin K enzyme in rodents has low homology with the human enzyme. Notwithstanding this, cathepsin K inhibitors have demonstrated potent antiresorptive activity when evaluated in established *in vivo* models of acute bone resorption, including the thyroparathyroidectomized (TPTX) rat model,²² and the OVX rat model.²³

Rabbit models

Given the interspecies sequence variation that exists in cathepsin K, a rabbit model has been established to evaluate cathepsin K inhibitors. While the rodent cathepsin K enzyme is only 88% homologous to the human enzyme, the sequence in the rabbit is 96% identical to human cathepsin K, and the monkey enzyme is identical to human.²⁴ Cathepsin K inhibitors have demonstrated pharmacodynamic activity with increased bone mineral density (BMD) in both the OVX rabbit²⁵ and "rabbit Schenk" models. In the recently validated rabbit Schenk assay, which imitated the growing rat model, cathepsin K inhibitors have yielded increases in BMD in a dose-dependent manner, with efficacy comparable with that of alendronate.²⁵

Non-human primates

The OVX rhesus monkey, an estrogen deficiency bone loss model characterized by accelerated bone resorption and formation, has been used to characterize bone active compounds. When selective cathepsin K inhibitors are administered to OVX rhesus monkeys, robust suppression of biochemical markers of bone resorption is observed. Oral dosing of the potent, selective, benzamide-containing aminonitrile, L-006235 to OVX rhesus monkeys once daily for 7 days results in a dose-dependent

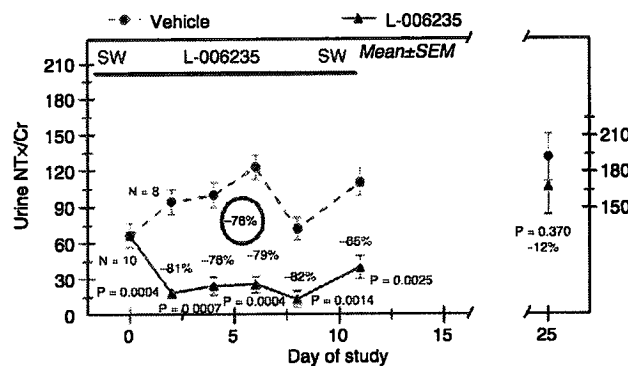


Figure 2 Ratio of N-telopeptide (NTx) to creatinine (Cr) during dosage (15 mpk, p.o., q.d.) of L-0006235 in adult OVX rhesus monkeys. Reprinted with permission from J. Med Chem., 2005, 48, 7520-7534.

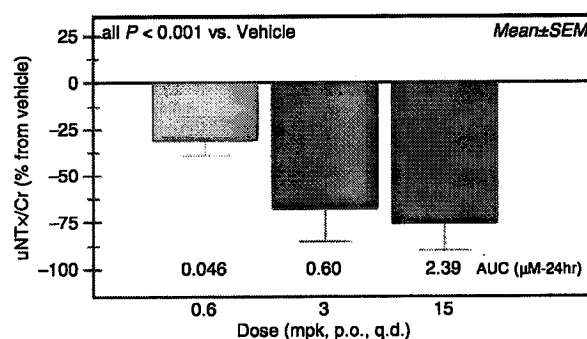


Figure 3 Dose-response effect of L-0006235 on urine NTx/Cr in the OVX monkey model. Reprinted with permission from J. Med Chem., 2005, 48, 7520-7534.

decrease in indices of bone resorption (Figures 2 and 3).²⁶ Similar decreases have also been observed with the administration of another cathepsin K inhibitor, relacatib (SB-462795), which decreases bone resorption and increases blood PTH levels.²⁷

Human studies

Although there are currently no approved cathepsin K inhibitors, proof of concept for the mechanism has been established. At least two cathepsin K inhibitors, odanacatib (MK-0822) and balicatib (AAE581), have demonstrated pharmacodynamic activity based on reductions in bone resorption markers and increases in BMD in humans. Three doses of odanacatib (0.5, 2.5, and 10 mg) were administered once daily to 30 postmenopausal female subjects ($N = 10$ per group (8 active, 2 placebo)) for 21 days. Robust reductions in urine N-telopeptide (uNTx; ~80%) were seen following daily administration of 2.5 and 10 mg of odanacatib for 21 days, whereas more modest reductions in uNTx (~15%) were seen following administration of 0.5 mg for 21 days.²⁸ The effects of odanacatib on serum CTx (sCTx) were similar to those seen with uNTx, eliciting a response of ~15, ~70, and ~80% reduction following 21 days of dosing odanacatib at 0.5, 2.5, and 10 mg, respectively. No significant effect was seen on bone-formation markers such as bone-specific alkaline phosphatase and osteocalcin.²⁸ Since osteoporosis therapies have pioneered the paradigm of

less-frequent dosing (once weekly, once monthly, and even twice yearly), and given the long pharmacokinetic half-life of odanacatib, it has also been evaluated in a once-weekly dosing paradigm in 48 postmenopausal women. Four panels of 12 postmenopausal female subjects (9 active, 3 placebo) were administered 5, 25, 50, and 100 mg odanacatib or placebo once weekly for 3 weeks.²⁹ Robust reductions in serum CTx and urine NTx (~50%) at trough (C168 h) were seen following weekly administration of 25, 50, and 100 mg in postmenopausal women for 3 weeks.²⁹ More recently, data from a multicenter, randomized, placebo-controlled trial have been presented that demonstrates increases in lumbar spine (LS) and femoral neck BMD following 12 months of dosing in 399 postmenopausal women (64.2 ± 7.8 years) with low BMD (mean T-scores ≤ -2.0 and ≥ -3.5) at LS, femoral neck, trochanter, or total hip.³⁰ Treatment resulted in dose-dependent increases in BMD from baseline. The highest dose of odanacatib evaluated (50 mg) resulted in a 3.4% increase in LS BMD, a 2.5% increase in femoral neck BMD, and a 58% reduction in uNTx/Cr.³⁰ Odanacatib was shown to be generally safe and well tolerated with no dose-related trends in adverse experiences.²⁹ Rash was reported in 8.4 and 3.8% of those in the placebo and 50-mg arms, respectively. Among those participants in the placebo arm, nine discontinued owing to adverse experiences, and four owing to adverse experiences in the 50-mg odanacatib arm.

In a 12-week randomized-controlled clinical trial performed in 140 postmenopausal women (N = 28/group), patients were randomized to daily oral doses of balicatib (5, 10, 25, or 50 mg) or placebo.³¹ A dose-dependent reduction in uNTx from baseline of -26.5, -48.0, -49.9, and -59.0% was observed for the 5, 10, 25, and 50 mg doses of balicatib, respectively. The demonstration of a favorable effect of balicatib on BMD has also been reported.³² In a multicenter, randomized, placebo-controlled, 12-month study, 675 postmenopausal women (mean age 62 years) with LS BMD T-score less than -2, were treated with 5, 10, 25, or 50 mg balicatib daily or placebo. Balicatib increased LS BMD (1.20, 3.16, 4.41, and 4.46% for the 5, 10, 25, and 50 mg doses, respectively, vs. 0.25% for placebo, P < 0.0005 for the 10, 25, and 50 mg groups vs. placebo)³². Balicatib also increased hip BMD (0.07, 1.77, 2.21, and 2.25% for the 5, 10, 25, and 50 mg doses, respectively, vs. 0.29% for placebo, P < 0.0005 for the 10, 25, and 50 mg groups vs. placebo).³² A dose-dependent reduction in bone resorption markers with little effect on serum bone-formation markers (osteocalcin, bone-specific alkaline phosphatase, and N-terminal propeptide of type I collagen (sP1NP)) were observed. A higher incidence of skin adverse experiences (mainly pruritus) was observed. A small number of patients experienced scleroderma/morphea-like skin changes which improved after discontinuing therapy.³²

Prior to the recent clinical experience with cathepsin K inhibitors, the only data available on cathepsin K deficiency in humans, were the findings from patients with pycnodysostosis. These individuals (both children and adults) also tend to have a reduction in bone resorption markers (uNTx, sCTx). In a report in 6 affected adults, uNTx measured 16 ± 8 vs. 37 ± 16 nmol/l bone collagen equivalents mmol/l creatinine in controls, P < 0.01, and

was significantly reduced in 17 affected children (P < 0.0003).³³ No abnormality was seen in markers of bone formation, osteocalcin, or carboxy-terminal propeptide of type I collagen (PICP), suggesting normal osteoblast activity.³³ Bone biopsies obtained from patients with pycnodysostosis reveal disturbances in bone remodeling³⁴ which may contribute to the increase in fragility fractures reported in patients with pycnodysostosis. However, the relevance of these findings in pycnodysostosis patients to any potential impact on bone strength in patients with osteoporosis treated with cathepsin K inhibitors is not known. It is clear that patients with pycnodysostosis have a life-long total absence of cathepsin K—a scenario quite different from the partial pharmacological inhibition in patients treated with cathepsin K inhibitors for osteoporosis.

CONCLUSIONS

In summary, the evolution of cathepsin K as a tractable osteoporosis target highlights the astute clinical observation of a high bone mass phenotype (pycnodysostosis), elegant science with the validation of potent and selective inhibitors in predictive models, and ultimately the inhibition of bone resorption with increased bone mass in patients with osteoporosis.

ACKNOWLEDGMENT

The authors are employees of Merck. The authors thank Dr Cameron Black for helpful comments in the preparation of this manuscript.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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ASUNTO	Radicación	04085271
	Trámite	002
	Evento	001
	Actuación	430
	Oficio	9665
	Folios	004

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

Documentos estudiados

Descripción: Folios: 22 a 220 como se radicó inicialmente.

Reivindicaciones: Folios: 221 a 258 como se radicó inicialmente.
Folios: 392 a 427 Modificado

Prioridad: Folios 1 a (5 de Marzo de 2002,US60/361,818).
(9 de Septiembre de 2002,US60/408,704).

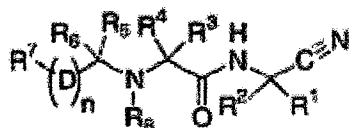
Respuesta del solicitante: Folios 370 a 390 y 428 a 460

Objeto de la invención

- Título de la solicitud: Inhibidores de la cistein-proteasa cathepsina.

El objeto de la presente solicitud de patente de invención consiste en:
Compuestos de N-cianometilamida derivados .

Reivindicaciones 1-13 : Compuesto de la fórmula (I) :



Reivindicación 14 : Composición farmacéutica del compuesto de fórmula I.
Reivindicación 15 : Composiciones farmacéuticas del compuesto de fórmula I en combinación con otros agentes.

El problema planteado en esta solicitud es la necesidad de compuestos de N-cianometilamida derivados como inhibidores de la cistein proteasa, incluyendo , inhibidores de captesinas K ,L, S y B y que son útiles en el tratamiento de osteoporosis.

Para solucionar este problema técnico la solicitud en estudio proporciona compuestos de fórmula (I), composiciones farmacéuticas que emplean los compuestos de fórmula (I) y procedimientos de preparación de dichas composiciones farmacéuticas.

El objeto de la invención definido anteriormente se clasificó de acuerdo con la Clasificación Internacional de Patentes (IPC) : C07D295/12; A61K31/275; A61K31/381, A61P19/10, C07C255/25.

De la solicitud de Patente

Reivindicaciones (artículo 30 D 486)

Para la reivindicación 1 se encuentran términos no concisos como :

- Para R1 , R2 , D, R9, Ra, Rb se encuentra el término , heterociclilo: No se especifica el número de miembros ni los heteroátomos que lo conforman.
- Para cuando R3 y R4 o R4 y R8 o Ra y Rb o Rc y Rd forman el anillo heterociclilo no especifica los heteroátomos que puede tener.
- Se encuentran términos como hidroxialquilo, alcoxi, haloalquilo, arilalquilo, heteroarilalquilo, haloalcoxi : No se especifica para la parte alquilica el número de átomos de carbono que puede tener.

Para las reivindicaciones 1, 5 y 8 se encuentra no son claras ni concisas porque :

- Cuando se define arilo " o bicíclico estable de hasta 12 átomos en cada anillo?.....", y cuando se define a heteroarilo " o tricíclico o de hasta 10 átomos en cada anillo? .

No se delimita adecuadamente en que rango de átomos de carbono o miembros que conforman los anillos. Se debe colocar como por ejemplo si fuera el caso de arilo de 6 a 12 átomos de carbono . Lo mismo para los heteroarilo.

En la reivindicación 1 se encuentran los términos :

- Para cuando R3 y R4 se unen para formar un "C₅₋₈ cicloalquenilo" .
- Para cuando Ra y Rb o Rc y Rd se unen para formar un "C₃₋₈ heterociclilo " .

Los cuales no son claros por un orden incorrecto en la traducción . Lo correcto es cicloalquenilo C₅₋₈ y heterociclilo C₃₋₈.

- También dentro de la reivindicación 1 se encuentra cuando se define a D, Ra, Rb que cuando se habla de que los grupos heteroarilo o heterociclilo "..... pueden estar opcionalmente sustituidos sobre el carbono o el heteroátomo? con uno.....". No es claro como todos los heteroátomos (entre ellos el oxígeno o el Azufre) pueden estar opcionalmente sustituidos.
- En la reivindicación 1 se habla de los sustituyentes Ra y Rb . Sin embargo dentro de la reivindicación no se encuentran grupos que los contengan.
- La reivindicación 15 , no es clara ni concisa ya que existen múltiples posibilidades de composiciones farmacéuticas obtenidas de la combinación de "los compuestos de la fórmula I con otros agentes como los descritos. Adicionalmente este Despacho no encuentra sustento técnico dentro de la descripción de dichas combinaciones; no hay evaluación sobre su interacción, co-acción, por ejemplo si crean sinergismo etc., . Por lo tanto se le sugiere al solicitante eliminar dicha reivindicación.

Respuesta a los argumentos del solicitante :

Se aceptan los argumentos expuestos por el solicitante de los folios 370 a 390 y los documentos aportados de los folios 428 a 460, en donde se evidencia la unidad inventiva , la novedad y el nivel inventivo de la solicitud. Sin embargo la actual solicitud para su nuevo capítulo reivindicatorio, aún presenta deficiencias por claridad y concisión tal como se evidenció anteriormente las cuales deben ser superadas.

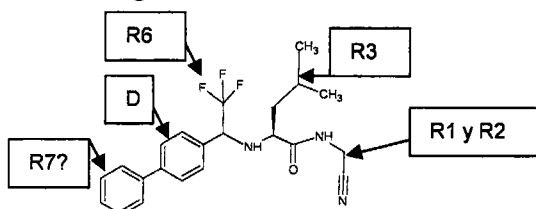
En cuanto a las reivindicaciones 9-13 a pesar que figuran como independientes encontramos que los compuestos que allí se divulgan a pesar que están debidamente sustentados en la descripción no son congruentes con la reivindicación 1 que es la que define el objeto de la invención. Esto se demuestra a continuación .

Por ejemplo uno de los compuestos que divulga dicha reivindicación corresponde al :

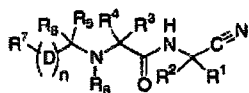


leucinamida:

y presenta la siguiente estructura :



Si observamos la reivindicación 1 encontramos que se reclama el compuesto de



fórmula .

Donde : R1 , R2, R4, R5 y R8 son hidrógeno.

R3 es alquilo C₁₋₆, (2-metilpropilo)

R6 es haloalquilo.

D es arilo . n es 1

R7 es como se define en el folio 393.

Sin embargo como se puede observar en el correspondiente folio con las nuevas modificaciones, R7 no se define como un grupo arilo y el compuesto no estaria dentro del alcance de la reivindicación que define la invención (1).

Por lo tanto se le sugiere y recomienda al solicitante restringir los compuestos de la actual solicitud de acuerdo a lo divulgado en la reivindicación 9 o a las tablas divulgadas en la descripción de los folios 44 a 53 las cuales se encuentran descritas de manera clara y concisa y a la cuales este Despacho reconoce el cumplimiento de los requisitos de patentabilidad.

Conclusión:

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el artículo 8 de la resolución reglamentaria No. 00210 del 15 de enero de 2001, advirtiéndole que contra él no procede recurso alguno en la vía gubernativa.


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha 21 AGO. 2009

CONCEPTO TECNICO No. 2588	EXAMINADOR TECNICO Código No. 202075
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