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MF

Trámite: 11
Evento: 378
Actuación: 523

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-258872-00006-0000

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Act. 523 RESPUESTA Folios: 36

Re: PI.-F.HOFFMANN-LA ROCHE AG y SIENA BIOTECH S.P.A.-Solicitud de
Patente de Invención en Fase Nacional PCT para "1,3-OXAZINAS COMO
INHIBIDORES DE LA BACE1 Y/O BACE2",-Clase C07D 265/08-
Expediente número 13-258.872-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 25 de noviembre de 2014.

2. Con el fin de responder las objeciones expuestas por el Señor Examinador en su Concepto Técnico correspondiente al Oficio No. **14551** en relación con la excepción a la Patentabilidad, las Reivindicaciones relativas a un uso, la claridad del Capítulo Reivindicatorio, y la Novedad y el Nivel Inventivo de la presente solicitud, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos por medio de los cuales no sólo se superan completamente dichas objeciones sino que además se establece de manera incontrovertible la patentabilidad de la solicitud en estudio.

3. **CAPÍTULO REIVINDICATORIO**

3.1. **Modificaciones al Capítulo Reivindicatorio**

En primer lugar, me permito manifestar que es deseo del solicitante presentar un **capítulo reivindicatorio modificado**, que reemplaza al actualmente presentado, con el propósito de superar las objeciones presentadas como parte del concepto técnico realizado por ese Despacho.

En conexión con el mencionado **capítulo reivindicatorio modificado**, me permito resaltar que éste no constituye de ninguna manera una ampliación al alcance de la solicitud, tal como fue originalmente presentada, toda vez que define los novedosos compuestos derivados de [1,3]-oxazinas con actividad inhibitoria de la BACE1 y/o BACE2 y sus respectivas composiciones farmacéuticas, los cuales son el objeto de la presente invención de acuerdo con las enseñanzas de la descripción. Dichas modificaciones se exponen a continuación:

- **Nueva Reivindicación 1:** tiene como base la combinación de las reivindicaciones 1 y 5 actualmente presentadas, y ha sido modificada en las definiciones del sustituyente Y de la fórmula I y de los términos *arilo*, *heteroarilo* y *heterociclilo* para restringir el alcance de la materia reclamada. Dichas modificaciones encuentra pleno sustento en la página 25 líneas 3 y 4, página 10 líneas 22 a 27, página 11 líneas 1 a 27, y página 13 líneas 3 a 6.
- **Nueva Reivindicación 2:** tiene como base la reivindicación 2 actualmente presentada, y ha sido modificada en las definiciones de los términos *arilo*, *heteroarilo* y *heterociclilo* para restringir el alcance de la materia reclamada. Dichas modificaciones encuentra pleno sustento en la página 10 líneas 22 a 27, página 11 líneas 1 a 27, y página 13 líneas 3 a 6.
- **Nuevas Reivindicación 3:** corresponde con la reivindicación 3 del capítulo actualmente presentado.
- **Nueva Reivindicación 4:** tiene como base la reivindicación 4 actualmente presentada, y ha sido modificada en la definición del término *arilo* para restringir el alcance de la materia reclamada. Dicha modificación encuentra pleno sustento en la página 13 líneas 3 a 6.
- **Nueva Reivindicación 5:** tiene como base la reivindicación 6 actualmente presentada, y ha sido modificada en la definición del término *heteroarilo* para restringir el alcance de la materia reclamada. Dicha modificación encuentra pleno sustento en la página 10 líneas 22 a 27.
- **Nuevas Reivindicación 6:** corresponde con la reivindicación 7 del capítulo actualmente presentado.

- **Nuevas Reivindicaciones 7 a 9:** tienen como base las reivindicaciones 8 a 10 actualmente presentadas, y se han eliminado de sus enunciados los compuestos de los ejemplos 34, 35, 36, 37, 39, 40, 41, 55, 57, 58, 59, 62, 63, 64 y 65 para limitar el alcance de la materia reclamada.
- **Nuevas Reivindicación 10:** corresponde con la reivindicación 14 del capítulo actualmente presentado.

De acuerdo a lo anterior, me permito señalar que la reivindicaciones **11, 12 y 13** del Capítulo Reivindicatorio actualmente presentado fueron **eliminadas** del documento modificado. Asimismo, me permito aclarar que el orden de enumeración y dependencia de las nuevas reivindicaciones se ha reajustado de conformidad con las modificaciones previamente descritas.

De esta forma, **el capítulo reivindicatorio modificado queda constituido por 10 reivindicaciones**, las cuales se refieren de manera más clara y concisa a las características técnicas esenciales de los compuestos derivados de [1,3]-oxazin-2-ilaminas que son objeto de la presente solicitud, y que cuentan con pleno sustento en las enseñanzas de la descripción.

3.2. Claridad del Capítulo Reivindicatorio

Ahora bien, con respecto a la claridad del capítulo reivindicatorio, el señor Examinador afirma lo siguiente:

• *"El capítulo reivindicatorio carece de concisión porque las definiciones de los sustituyentes conllevan a un número indefinido de compuestos que no encuentran soporte en la descripción. Los términos arilo, heteroarilo y heterociclilo, a los que no se les menciona el número de átomos que los componen, corresponden a definiciones genéricas que no permiten distinguir la invención de los compuestos conocidos en el estado de la técnica. Se sugiere a la solicitante restringir la solicitud a los compuestos sustentados en la descripción (...)"*.

Respecto al anterior señalamiento, me permito indicar que, tal y como se expuso en el numeral 3.1 del presente memorial, las nuevas reivindicaciones 1, 2, 4 y 5 han sido modificadas de tal forma que las definiciones del sustituyente Y de la fórmula I y de los términos *arilo*, *heteroarilo* y *heterociclilo* de los sustituyentes X, Z y R⁸ han sido restringidas en su alcance. De este modo, las anteriores definiciones se leen como sigue:

- ✓ Y se elige entre el grupo formado por $-NHCH_2-$, $-NH-$, o está ausente.
- ✓ Arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente que tiene de 6 a 10 átomos de carbono en el anillo.
- ✓ Heteroarilo se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático.
- ✓ Heterociclilo se define como un sistema cíclico monovalente saturado o parcialmente insaturado, de 4 a 9 átomos en el anillo, que contiene 1, 2 ó 3 heteroátomos elegido entre N, O y S, donde los demás átomos del anillo son carbonos, y en donde bicíclico indica que está formado por dos ciclos que tienen en común dos átomos del anillo.

Ahora, de acuerdo a las anteriores restricciones, los compuestos reclamados en las nuevas reivindicaciones 7 a 9 fueron reducidos a un número de compuestos que encuentran pleno sustento en la descripción y que reflejan las nuevas limitaciones del capítulo reivindicatorio. Los siguientes compuestos fueron eliminados de dichas reivindicaciones:

- Compuesto del Ejemplo 34: *4-[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-fenoximetil]-benzonitrilo.*
- Compuesto del Ejemplo 35: *(R)-4-[3-(4-cloro-benciloxi)-fenil]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.*
- Compuesto del Ejemplo 36: *(R)-5,5-difluor-4-metil-4-[3-(4-trifluormetil-bencil-oxi)-fenil]-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.*
- Compuesto del Ejemplo 37: *(R)-5,5-difluor-4-metil-4-[2-(4-metil-bencil)-3H-benzo-imidazol-5-il]-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.*
- Compuesto del Ejemplo 39: *(R)-5,5-difluor-4-[5-fluor-2-(4-metoxi-bencil)-1H-indol-6-il]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.*

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- Compuesto del Ejemplo 40: *(R)*-5,5-difluor-4-[5-fluor-2-(4-metil-bencil)-3H-indol-6-il]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.
- Compuesto del Ejemplo 41: 3-((*R*)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-N-prop-2-inil-benzamida.
- Compuesto del Ejemplo 55: *(R)*-4-[2-(4-etil-bencil)-3H-benzoimidazol-5-il]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.
- Compuesto del Ejemplo 57: *(R)*-4-[5-((*RS*)-2,2-difluor-ciclopropilmetoxi)-fenil]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.
- Compuesto del Ejemplo 58: *(R)*-5,5-difluor-4-metil-4-[3-(3,3,3-trifluor-propoxi)-fenil]-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.
- Compuesto del Ejemplo 59: *(R)*-4-(3-ciclopropilmetoxi-fenil)-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina.
- Compuesto del Ejemplo 62: (3-cloro-quinolin-8-il)-amida del ácido (1*S*,2*S*)-rel-2-((*R*)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-ciclopropanocarboxílico.
- Compuesto del Ejemplo 63: (3-cloro-quinolin-8-il)-amida del ácido (1*R*,2*R*)-((*R*)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-ciclopropanocarboxílico.
- Compuesto del Ejemplo 64: (3-cloro-quinolin-8-il)-amida del ácido (1*S*,2*S*)-((*R*)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-ciclopropanocarboxílico.
- Compuesto del Ejemplo 65: (6-ciano-tieno[3,2-*b*]piridin-3-il)-amida del ácido (1*R*,2*R*)-2-((*R*)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-ciclopropanocarboxílico.

A la luz de lo anterior, es evidente que el nuevo capítulo reivindicatorio constituye la generalización del tipo de compuestos que han sido sintetizados y define de manera clara y concisa el sustituyente Y del compuesto de formula I y los términos *arilo*, *heteroarilo* y *heterociclilo*, con lo cual se permite distinguir la invención de los compuestos conocidos en el arte previo. Es así, que me permito manifestar que se supera completamente la objeción realizada por el señor Examinador a éste respecto.

• *"La reivindicación 11 no es clara porque no se especifican las condiciones de reacción en que se debe llevar a cabo el proceso reclamado ni tampoco cuales grupos aminoprotectores se utilizan (...). Adicionalmente, no se especifica cuáles son las reacciones que se deben llevar a cabo para obtener el compuesto de fórmula I a partir del compuesto de fórmula I. Se requiere al solicitante hacer las aclaraciones necesarias".*

Sobre este particular me permito señalar que, tal y como se expuso en el numeral 3.1 del presente memorial, la reivindicación 11 ha sido eliminada del capítulo reivindicatorio.

En vista de lo discutido anteriormente, me permito resaltar que el nuevo capítulo reivindicatorio es totalmente claro y conciso al reclamar compuestos derivados de 1,3-oxazina con actividad inhibitoria de BACE1 y/o BACE2. Asimismo, dicho capítulo modificado supera completamente las objeciones de claridad presentadas por el Señor Examinador y, por lo tanto, cumple a cabalidad con lo estipulado por el Artículo 30 de la Decisión Andina 486 de 2000

4. REIVINDICACIONES RELATIVAS A USOS O SEGUNDOS USOS.

En relación a esta objeción, el Señor Examinador establece que la reivindicación 12 indica el usos que puede darse al compuesto aunque la reivindicación esté encabezada en términos de *"el compuesto (...)"*. En este sentido, dicha reivindicación no es patentable de acuerdo a los Artículos 14 y 21 de la Decisión 486/2000.

Tomando en consideración la anterior objeción, me permito aclarar que, de acuerdo con las modificaciones expuestas en el numeral 3.1 del presente memorial, la reivindicación 12 ha sido eliminada.

5. EXCEPCIONES A LA PATENTABILIDAD.

Por otra parte, el Señor Examinador indica que la reivindicación 13 no sería patentable al referirse a un método de tratamiento, de acuerdo con el Artículo 20 literal (d) de la Decisión 486/2000.

Sobre este particular, me permito señalar que tal y como se expuso en el numeral 3.1 del presente memorial, dicha reivindicación 13 también ha sido eliminada y no hace parte del nuevo capítulo reivindicatorio.

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En este sentido, me permito señalar que el mencionado capítulo reivindicatorio modificado se ajusta completamente a los requerimientos del Artículo 20 de la Decisión Andina 486 de 2000.

6. NOVEDAD.

Ahora bien, el Señor Examinador asegura que las reivindicaciones 1 a 4, 6, 7, 11 y 14 en estudio no cumplen con el requisito de novedad en vista de las enseñanzas del documento **CO 12-113.850 (D1)**.

En este sentido, el Señor Examinador asegura que las características técnicas esenciales del compuesto derivado de [1,3] oxazin-2-ilaminas reclamado en la reivindicación 1 del capítulo actualmente presentado habían sido divulgadas previamente en el documento D1.

De acuerdo a las tablas comparativas del Concepto Técnico, las características técnicas de la invención descritas en la reivindicación 1 ya se enseñaban de manera independiente en las anterioridades del documento D1. Por lo tanto, el señor Examinador concluye que el compuesto de fórmula I, derivado de [1,3]oxazinas y con actividad inhibitoria para BACE1 y/o BACE2, ya era conocido en el estado de la técnica.

Adicionalmente, el señor Examinador asegura que las reivindicaciones dependientes 2 a 4, 6, 7, 11 y 14 no mencionan características técnicas adicionales que puedan brindarle novedad al compuesto reclamado en la reivindicación 1.

En respuesta a lo anterior, inicialmente me permito reiterar que, de conformidad con el numeral 3.1 del presente memorial, la reivindicación 11 actualmente presentada ha sido eliminada del nuevo capítulo reivindicatorio.

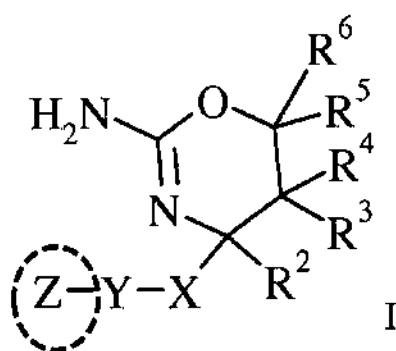
Por su parte, con respecto a la reivindicación 1 y sus respectivas dependencias (*que corresponden a la nueva reivindicación 1*), **me permito señalar que dicho documento D1 no sugiere la misma fórmula estructural ni las mismas definiciones de los sustituyentes del compuesto de fórmula I de la invención, tal y como es reclamado dentro del nuevo capítulo reivindicatorio que se presenta junto con este memorial.**

En primer lugar y de acuerdo con las modificaciones hechas al capítulo reivindicatorio expuestas en el memorial 3.1 del presente memorial, la nueva reivindicación 1 fue considerablemente restringida para proteger compuestos de fórmula I derivados de [1,3]oxazin-2-ilaminas con actividad inhibitoria para BACE1 y/o BACE 2.

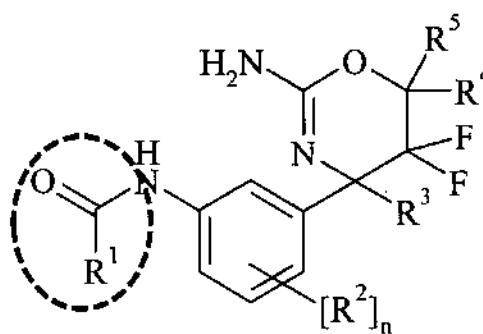
Ahora bien, tal y como describe el señor Examinador en su concepto técnico, el documento D1 efectivamente divulga "compuestos tipo 2-amino-5,5-difluor-5,6-dihidro-4H-[1,3]oxazinas-4-il)-fenil]-amida útiles como inhibidores de bace1 y/o BACE2".

Sin embargo, y detallando la estructura de dichos compuestos, los compuestos del documento D1 son derivados específicamente de [(2-amino-5,5-difluor-5,6-dihidro-4H-[1,3]oxazinas-4-il)-fenil]-amidas de ácidos carboxílicos sustituidos. En este sentido, me permito manifestar que el documento D1 **no enseña ni menciona en ninguna parte compuestos específicamente derivados de 5,6-dihidro-4h-[1,3]oxazina-2-ilaminas, los cuales son los objetos reclamados en la presente invención.**

De acuerdo con las enseñanzas del documento D1, todos los compuestos reclamados allí tienen un grupo carbonilo en la misma posición que corresponde al sustituyente Z de los compuestos de fórmula I de la presente invención. Al analizar detalladamente las definiciones para dicho sustituyente, se encontró que ninguno de los compuestos de fórmula I de la presente invención (*tanto los 66 ejemplos como las estructuras derivadas de los sustituyentes*) incluye, como sustituyente Z, un grupo funcional relacionado al grupo carbonilo (-CO), específicamente a las amidas (-CONR'-) o a los ácidos carboxílicos (-COOH), lo cual constituye una diferencia relevante entre los compuestos reclamados por la presente solicitud y el estado anterior de la técnica:

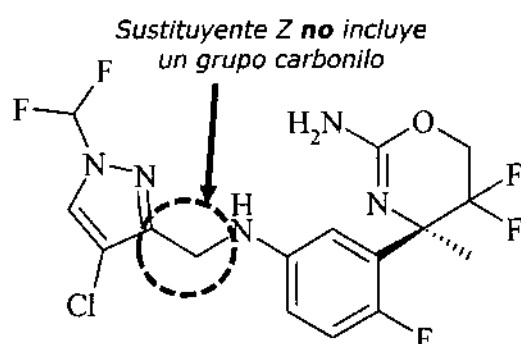


Fórmula I del compuesto derivado de [1,3]-oxazin-2-ilamina de la presente invención

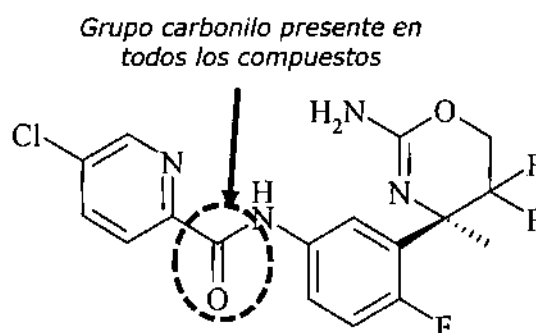


Fórmula I del compuesto derivado de [1,3]-oxazin-amida del ácido carboxílico del documento D1

Específicamente, dicha diferencia se evidencia con mayor claridad entre el Ejemplo 16 de la presente solicitud y el Ejemplo 1 del documento D1:



Compuesto del Ejemplo 16 de la presente invención

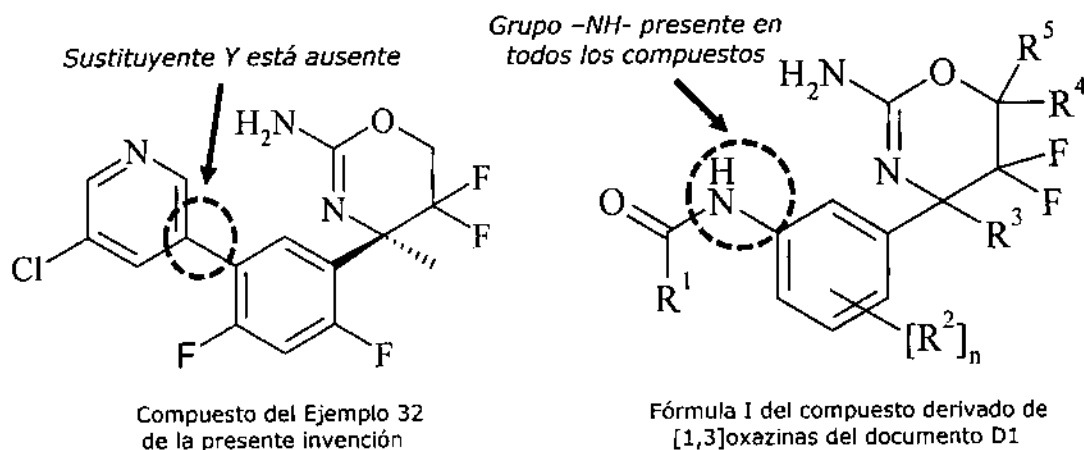


Compuesto del Ejemplo 1 del documento D1

Ante lo anterior, es claro que, mientras el documento D1 divulga amidas de ácidos carboxílicos sustituidos como se evidencia con la presencia del grupo carbonilo en todos los compuestos, la presente invención se enfoca en proteger compuestos derivados de aminas que en ningún caso incluyen un grupo carbonilo en su estructura.

Ahora, considerando las modificaciones hechas al capítulo reivindicatorio actualmente presentado, dichas restricciones también permiten diferenciar aún más los compuestos de la invención con respecto a los compuestos del arte previo, y por ende, contribuyen a la novedad de la presente solicitud.

Así, con respecto al sustituyente Y de los compuestos de fórmula I, dicho sustituyente quedó definido por los grupos funcionales $-NHCH_2-$ y $-NH-$ o queda ausente de la estructura. Al comparar con los compuestos de D1, se observó que la fórmula I incluye únicamente el grupo funcional $-NH-$ en la posición que corresponde al sustituyente Y de los compuestos de la presente invención. Es decir, mientras que los compuestos de la presente solicitud permiten definir dicho sustituyente con 3 opciones diferentes, los compuestos de D1 están supeditados a siempre incluir el grupo $-NH-$ en dicha posición. Tal diferencia se puede evidenciar con cualquiera de los Ejemplos 9 a 11, 15, 31 a, 33, 50 a 54B, 60 y 61, en donde el sustituyente Y está ausente:



En el mismo análisis de las diferencias entre los sustituyentes, se evidencia que no todos los compuestos de la presente invención tienen los 2 átomos de flúor en la posición 5 del anillo de la [1,3] oxazina de los compuestos del documento D1. Tal es el caso de los compuestos de los: a) Ejemplos 1, 2, 53 y 54B, donde no hay ningún átomo de flúor en dicha posición, b) Ejemplos 3 a 11 y 56, donde sólo hay un átomo de flúor en dicha posición, y c) Ejemplos 12 a 14, donde hay un átomo de flúor y un grupo metilo en dicha posición.

En este punto, considero pertinente aclarar que, si bien los compuestos de la presente invención comparten con los compuestos del documento D1 el anillo de la [1,3] oxazina, el resto de la estructura de los compuestos de la presente solicitud son sustancialmente diferentes, por lo que no es correcto afirmar que el objeto de la dicha solicitud no es novedoso a la luz del estado de la técnica anterior.

En vista de todo lo anterior, **es evidente que en el documento D1 no existe ninguna enseñanza o sugerencia que se refiera compuestos derivados de [1,3] oxazinas con exactamente la mismas características estructurales atribuidas a los compuestos de fórmula I reclamados en la presente solicitud.**

Ahora bien, en el contexto de las patentes es claro que para que un documento afecte la novedad de una solicitud, éste debe revelar, de manera explícita y específica, todas y cada una de las características de la invención interactuando de la misma manera que lo hacen en la solicitud. De acuerdo con la Guía para el examen de patentes de invención y modelo de utilidad de la Superintendencia de Industria y Comercio, el señor Examinador debe "verificar (...) si la invención reivindicada es idéntica a lo revelado en el estado de la técnica, en cuyo caso se considera que no tiene novedad".

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Visto lo anterior, es absolutamente claro que **los compuestos derivados de [1,3] oxazin-2-ilaminas reclamados en la nueva reivindicación 1 son totalmente novedosos** frente a las enseñanzas del documento D1 porque dicha anterioridad de ninguna manera divulga compuestos inhibitorios de BACE1 y/o BACE2 con exactamente los mismos sustituyentes en su estructura química.

Así, es evidente que los posibles sustituyentes Z del compuesto de fórmula I de la invención son completamente diferentes al grupo carbonilo encontrado en la misma posición en los compuestos del documento D1. Asimismo, es claro que no todos los compuestos de la invención están limitados a tener un grupo -NH- en la posición del sustituyente Y ni a incluir dos átomos de flúor en la posición 5 del anillo de [1,3] oxazina, como es el caso de todos los compuestos de D1.

En consecuencia, **me permito afirmar ante ese Despacho que los compuestos derivados de [1,3]oxazin-2-ilaminas con actividad inhibitoria de BACE 1 y/o BACE 2 reclamados en la nueva reivindicación 1 y sus respectivas reivindicaciones dependientes presentan un inequívoco carácter novedoso frente a las enseñanzas del documento D1**, y por lo tanto, cumplen a cabalidad con los requisitos de patentabilidad establecidos en el artículo 16 de la Decisión 486 de 2000.

7. **NIVEL INVENTIVO.**

Finalmente, el Señor Examinador considera que la materia reclamada en la reivindicación 5 actualmente presentada carecería de altura inventiva con respecto a las enseñanzas de los documentos **CA 2683887 (D2)** y **Moreira Lima, L. y Barreiro, E. J. "Bioisosterism: a useful strategy for molecular modification and drug design". Current Medicinal Chemistry, 2005. Vol 12m p. 23-49 (D3).**

En conexión con lo anterior, el Señor Examinador asegura que la persona del oficio normalmente versada en la materia, basándose en la relación bioisostérica existente entre los grupos -O- y -S- divulgada en el documento D3, remplazar el anillo de oxazina por el anillo de dihidrotiazina divulgado en el documento D2, conservando el efecto terapéutico, y así llegar al objeto de la reivindicación 5 de la solicitud.

Sin embargo, sobre esta particular objeción **me permito señalar que las enseñanzas de los mencionados documentos D2 y D3, solas o en combinación, de ninguna manera pueden conducir a las estructuras particulares de los compuestos de la invención, tal y como se reclaman dentro del capítulo reivindicatorio modificado que se presenta junto con este memorial.**

Efectivamente, el documento D2 revela compuestos derivados de dihidro-[i,3]tiazinas con actividad inhibitoria de BACE1 y útiles en el tratamiento de enfermedades inducidas por la producción, secreción y/o depósito de la proteína β amiloide. A pesar de que dichos compuestos comprenden una porción de su estructura que es similar a la de los compuestos de la presente invención, la sustitución de la misma con los diferentes sustituyentes sugeridos conduce a un gran número de compuestos con una estructura significativamente diferente a la de los compuestos de la presente invención.

En este sentido, es importante resaltar que el documento D2 da 702 ejemplos que combinan los diferentes radicales de la molécula de fórmula I de distintas maneras. Para analizar las diferencias entre las características estructurales de los compuestos de D2 y de la presente invención, es claro que el experto en la materia se concentraría en aquellos ejemplos que presentan datos biológicos, por lo que, se compararon los ejemplos de la descripción con mejores valores de IC_{50} . En este sentido, se toman como referencia las Tablas 139 a 141 de las páginas 162 y 163 de D2, las cuales incluyen los distintos valores de iguales o menores a 1 μM para ejemplos seleccionados.

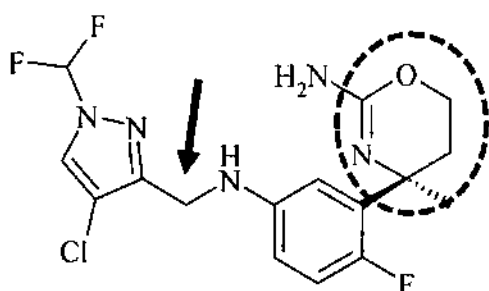
Así, se tiene que los compuestos con mayor potencia inhibitoria para BACE1 divulgados en el documento D2 son los compuestos de los Ejemplos 655, 669 y 674, los cuales presentan un valor de IC_{50} de 0.01 μM , seguidos muy de cerca por los compuestos de los Ejemplos 468 y 551 con valores IC_{50} de 0.02 μM .

En este orden de ideas, cabe destacar que los 3 ejemplos de D2 mencionados anteriormente muestran un patrón de sustitución similar. Específicamente los 4 ejemplos **presentan, como parte de su estructura, el anillo de dihidro-[i,3]tiazina, un grupo metilo en la posición 4 del anillo de tiazina, y un grupo carbonilo que, junto a la unión con el grupo amino de la estructura, generan que el compuesto sea un derivado de la amida de un ácido carboxílico.**

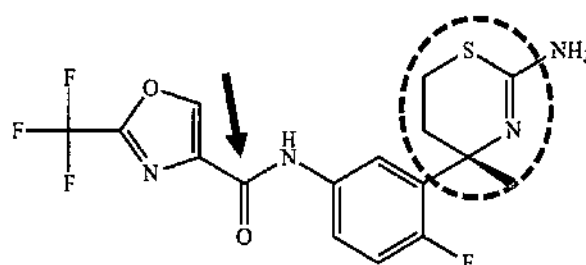
Igualmente, se observa que la realización preferida para los compuestos del documento D2 incluye, además del anillo de [1,3] tiazina, el grupo carbonilo, por lo que todos los compuestos son derivados de la amida del ácido carboxílico sustituido. De acuerdo a lo discutido en el numeral 6 del presente memorial, dicho grupo funcional no se encuentra en ningún compuesto de la invención, lo que resulta en una diferencia relevante del objeto de la invención frente a los compuestos del estado del arte previo.

En síntesis, los compuestos reclamados en la presente solicitud difieren de las enseñanzas divulgadas en el documento D2 en dos aspectos importantes:

- i) **los compuestos de la invención presentan un grupo oxazinilo (ver círculo en rojo), mientras que los compuestos divulgados en D2 presentan como parte fundamental de su molécula un grupo tiazinilo (ver círculo en rojo), y**
- ii) **los compuestos de la invención no definen ningún grupo carbonilo para el sustituyente Z (ver flecha azul) mientras que todos los compuestos divulgados en D2 incluyen dicho grupo carbonilo unido a un grupo amino (ver flecha azul), creando así una amida en la estructura.**



Compuesto del Ejemplo 1 (IC₅₀: 0.013)
de la presente invención



Compuesto del Ejemplo 669 (IC₅₀: 0.01)
del documento D2

Lo anterior significa que la persona del oficio medianamente versada en la materia tendría que realizar dos tipos diferentes de modificaciones a la molécula de tiazinilo amino-sustituido de D2, para llegar a los compuestos que se reclaman en la presente solicitud. En este sentido, **el documento D2 no brinda ninguna sugerencia acerca de que estas modificaciones deben realizarse con el fin de llegar a obtener compuestos más adecuados.**

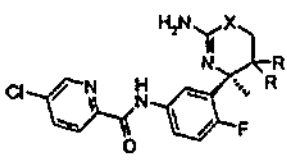
Cabe agregar que también existen otras diferencias que constituirían modificaciones extra para que una persona versada en la materia, basándose en las enseñanzas del documento D2, lograra obtener los compuestos de fórmula I con la misma estructura que se reclama en la presente solicitud. En este sentido, mientras que todos los 702 compuestos ejemplificados en D2 presentan un grupo funcional -NH- en la posición que corresponde al sustituyente Y de los compuestos de la presente invención, los compuestos de la presente solicitud permiten definir dicho sustituyente con 3 opciones diferentes, tal y como se exponen en el numeral 6 del presente memorial.

Ahora, en referencia al documento D3, la información allí relaciona conceptos sobre bioisosterismo y omite cualquier referencia a algún compuesto relacionado con el campo de la invención. Específicamente, divulga el reemplazo bioisostérico entre los grupos -S- y -O-, el cual, en la opinión del señor Examinador, motivaría a la persona versada en la materia a incluir dicho cambio para obtener los compuestos de la presente solicitud.

Sin embargo, me permito manifestar ante ese Despacho que el reemplazo del átomo de azufre por el átomo de oxígeno en la posición 1 del anillo de tiazina no es una acción obvia, puesto que la persona versada en la materia no podría esperar que la misma actividad inhibitoria se conserve al reemplazar dichos átomos.

En este sentido, y como un complemento a los argumentos presentados anteriormente, me permito referirme al estudio realizado por Hilpert et al., que se adjunta al presente memorial, el cual demuestra claramente que (ver cuadro 1 de la página 3984) las oxazinas di-fluoradas son las más adecuadas como inhibidores de BACE1, esto en comparación con oxazinas sin fluoración y con tiazinas con y sin sustitución con flúor:

Table 1. In Vitro and in Vivo Profiles of Oxazines 1a and 14a and Thiazines 112 and 113



compd	X	R	pK _a ^b	IC ₅₀ (nM)			shift BACE1 enzyme vs cell-based	brain Aβ40 reduction in mice at 30 mg/kg ^d (%)
				hBACE1 enzyme	hBACE2 enzyme	APP40 cell-based ^c		
1a	O	H	9.8	0.137	0.052	0.010	14	29
14a	O	F	6.3	0.026	0.015	0.017	2	69
112	S	H	9.0	0.016	0.009	0.001	16	92
113	S	F	6.1	0.014	0.010	0.004	3	0

^aIC₅₀ values are means of at least 2 independent experiments. ^bpK_a determined by capillary electrophoresis. ^cHEK293 cells transfected with wild-type human APP. ^dCompound given orally, n = 3-4, 4h post dose, mean reduction as compared to vehicle control.

Tabla 1. Documento Hilpert et al.

De acuerdo con la información consignada en la Tabla 1 de la página 3984 y tras la lectura atenta de la sección de resultados y discusión del artículo de Hilpert et al, se puede concluir que el impacto del reemplazo de un átomo de azufre por un átomo de oxígeno en el grupo principal sobre las actividades BACE1 y las eficacias in vivo es complejo y conduce a resultados inesperados.

Por lo tanto, a la luz de las divulgaciones dadas en el artículo de Hilpert et al, es completamente evidente que **el reemplazo del grupo tiazinilo por el grupo oxazinilo y la disustitución con flúor NO son eventos convencionales en el campo técnico.**

Igualmente, me permito señalar que desde el punto de vista estructural, de ninguna manera es predecible que un compuesto cuyo núcleo principal consiste en un oxazinilo y no en un tiazinilo, conserve su actividad como inhibidor de BACE 1 y/o BACE2, dado que en primer lugar **los remplazos bioisostéricos son altamente impredecibles**, tal y como lo señala el documento "Foye's Principles of Medicinal Chemistry"¹:

"Lo que puede ser un remplazo bioisostérico exitoso para una molécula dada interactuando con un receptor particular muy a menudo no tiene ningún efecto o elimina cualquier actividad biológica en otra. Así, el uso de remplazo bioisotérico (clásico o no clásico) en el diseño de medicamentos es altamente dependiente del sistema biológico que está siendo investigado. No existen reglas invariables para determinar qué remplazo bioisostérico va a funcionar en una molécula dada." (resaltado fuera de texto).

Ahora, a pesar de que documentos básicos en el campo técnico de la invención señalen que existen reemplazos bioisostéricos que podrían permitir tener compuestos que mantuvieran la actividad biológica original, la persona versada en la materia reconocería que existen muchas circunstancias en las cuales los reemplazos bioisostéricos resultan en una disminución de la actividad biológica del compuesto dependiendo de la manera en que este interactúe con la molécula biológica objetivo.

Es así como todos estos factores deben ser tenidos en consideración cuando se realizan modificaciones dentro de una molécula buscando un bioisótero, tal como lo

¹ Foye's Principles of Medicinal Chemistry, Página 54, 1er párrafo, parte I, 7ma edición, 2013

señala Thornber²: *"al hacer un reemplazo bioisostérico, los siguientes parámetros del grupo a ser cambiado pueden ser considerados: tamaño, forma (ángulos de enlace, hibridización), distribución electrónica (polarizabilidad, efectos inductivos, carga, dipolos), solubilidad lipídica, solubilidad acuosa, pK_a, reactividad química (incluyendo probabilidad de metabolismo), capacidad de uniones de hidrógeno (...) Es poco probable que un reemplazo bioisostérico deje todos estos parámetros sin perturbar".* (Resaltado fuera de texto).

En vista de todo lo anterior, es evidente que las enseñanzas del documento D3 de ninguna manera complementan las enseñanzas de D2 para llegar a los compuestos de la invención, mucho más si se tiene en cuenta que el documento D3 no hace mención alguna sobre compuestos derivados de oxazina con actividad inhibitoria de BACE1 y/o BACE2.

Consecuentemente, me permito afirmar que **se descarta cualquier indicio de obviedad en la sustitución del átomo de azufre por un átomo de oxígeno en el anillo de la tiazina y se evidencia el nivel inventivo de los compuestos derivados de 1,3-oxazin-2-ilaminas de la nueva reivindicación 1 de la presente solicitud.**

Ahora bien, el punto no es si un experto en la materia podría haber llegado a la invención mediante la adaptación o modificación de las enseñanzas del documento D2 mediante las enseñanzas divulgadas en D3, sino que si lo hubiera hecho de esa manera, ¿por qué el documento D3 le motivó a hacerlo con la expectativa de resolver el problema técnico objetivo?

Es más, **¿qué razón tendría la persona versada en la materia para que, a la luz del problema resuelto por la presente invención, decidiera modificar los compuestos de D2 de manera que las estructuras resultantes comprendan compuesto derivados de [1,3]-oxazin-2-ilamina, y no otro tipo de modificación?**

En conexión con lo anterior, muy respetuosamente me permito señalar que la única motivación que tendría la persona versada en la materia para hacer esta particular modificación sería la revelación hecha por la presente invención, por lo que el análisis de nivel inventivo realizado por el Señor Examinador está enmarcado dentro de lo que en el ámbito de las patentes se conoce como *"hindsight"* o análisis *"Ex post facto"*. Conociendo de antemano la invención, el Señor Examinador está

² Thornber, C. W. *"Isosterism and Molecular modification in drug design"* Chem. Soc. Rev. 1979, 8, 563-580.

seleccionando partes específicas de las enseñanzas contenidas dentro del documento D2, las cuales modifica de forma que permiten construir la materia reclamada por la reivindicación 1 de la presente solicitud. Dichas acciones o modificaciones de ningún modo podrían ser evidentes para la persona versada en la materia en el momento en el cual se desarrolló la invención.

En este sentido, vale la pena resaltar que, al tener conocimiento de la invención, es de esperarse que dichas modificaciones particulares de las enseñanzas de D2 parezcan obvias, toda vez que ya se sabe de antemano que efectivamente esta sustitución de -S- por -O- resuelve el problema planteado. Sin embargo, esta información no era conocida por el inventor en el momento de desarrollar la invención, por lo que no podría tener certeza de la utilidad de realizar estos pasos.

En vista de este señalamiento, y a la luz de lo discutido en el presente memorial, me permito resaltar que al no "*saltar a la vista de manera clara y directa*" a partir de la combinación de los documentos D2 y D3 una compuesto derivado de 1,3-oxazin-2-ilamina, como el considerado en la presente invención, y al no tenerse "*certeza clara y manifiesta*" de que dicho compuesto conservaría el efecto inhibitorio de BACE1 y/o BACE2, es claro que los documentos D2 y D2 no pueden de ninguna manera afectar el Nivel Inventivo de la solicitud en estudio.

8. RECONOCIMIENTO DE LA PATENTABILIDAD DE LA PRESENTE SOLICITUD EN OTRAS OFICINAS DE PATENTES.NIVEL INVENTIVO.

Finalmente y aparte de lo anteriormente mencionado, me permito señalar que la solicitud de patente en cuestión ha sido reconocida como nueva e inventiva en la opinión emitida por la Oficina de Patentes y Marcas de los Estados Unidos (USPTO) para la correspondiente en Estados Unidos **US 2012/0295900** (ver documento anexo).

Ahora bien, ante todo es preciso señalar que con la información mencionada anteriormente de ninguna manera pretendo desconocer la independencia de la que goza la Superintendencia de Industria y Comercio al momento de analizar los méritos de patentabilidad de una solicitud, de acuerdo con las disposiciones del Convenio de París para la protección de la propiedad industrial.

Sin embargo, sí considero de gran importancia resaltar el hecho de que la materia técnica revelada en la solicitud de patente en cuestión ha sido catalogada como **novedosa, inventiva e industrialmente aplicable** dentro de la opinión

escrita emitida por la USPTO, hecho que evidentemente representa un claro indicio de que la materia técnica revelada en la solicitud de patente en cuestión cumple a cabalidad con los requisitos de patentabilidad establecidos en la Decisión 486 de 2000.

9. **CONCLUSIÓN.**

Para finalizar, me permito reiterar que el capítulo reivindicatorio modificado presentado junto con este memorial es totalmente claro y conciso al reclamar los compuestos derivados de [1,3]-oxazin-2-ilaminas con actividad inhibitoria de BACE1 y/o BACE2, y está plenamente soportado dentro del texto de la descripción, cumpliendo así con lo exigido por la mencionada Decisión Andina 486 de 2000.

Por otro lado, en virtud de los argumentos discutidos con respecto al documento citados, se estableció de manera incontrovertible que dichos compuestos derivados de 1,3-oxazin-2-ilaminas con actividad inhibitoria de BACE 1 y/o BAC2, los cuales son objeto de la solicitud en estudio, de ninguna manera resultarían evidentes para la persona versada en la materia a partir de las enseñanzas del documento, demostrando irrefutablemente la patentabilidad de la presente solicitud.

10. Anexo al presente memorial, me permito presentar:

- i) Recibo número 15-007204, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de las modificaciones mencionadas;
- ii) Copia de las páginas **206 a 218** que corresponden al **capítulo reivindicatorio modificado**, tal como se discutió en el presente memorial, constituido por **10** reivindicaciones;
- iii) Artículo: Hilpert et al. " *β -Secretase (BACE1) Inhibitors with High in Vivo Efficacy Suitable for Clinical Evaluation in Alzheimer's Disease*". Journal of Medicinal Chemistry, 2013, 56, 3980–3995; y
- iv) Copia del *Notice of Allowance* de la USPTO correspondiente a la publicación americana de patente US 2012/0295900, la cual corresponde a la presente solicitud de patente.

JOSE LLOREDA CAMACHO & CO.

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11. En vista de 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,

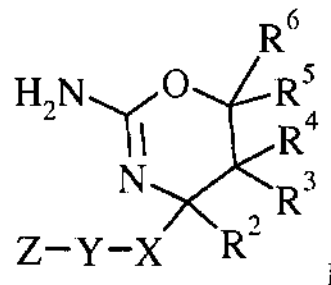


ALICIA LLOREDA RICAURTE

T. P. 53.215

REIVINDICACIONES

1. Un compuesto de la fórmula I:



en la que:

X se elige entre el grupo formado por:

- i) arilo, el cual se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,
- ii) arilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^1 , donde el arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,
- iii) heteroarilo, el cual se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático,
- iv) heteroarilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^1 y halógeno-arilo, donde el arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo, y el heteroarilo se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático,
- v) cicloalquilo C_{3-6} , y
- vi) cicloalquilo C_{3-6} sustituido por 1-2 sustituyentes elegidos individualmente entre R^1 ;

Y se elige entre el grupo formado por:

- i) $\text{-NHCH}_2\text{-}$,
- ii) -NH- , o
- iii) está ausente;

Z se elige entre el grupo formado por:

- 5 i) arilo, el cual se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,
- ii) arilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^8 , donde el arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,
- 10 iii) heteroarilo, el cual se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático,
- 15 iv) heteroarilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^8 , donde el heteroarilo se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático,
- 20 v) cicloalquilo C_{3-6} ,
- vi) cicloalquilo C_{3-6} sustituido por 1-2 sustituyentes elegidos individualmente entre R^8 ,
- vii) heterociclilo, el cual se define como un sistema cíclico monovalente saturado o parcialmente insaturado, de 4 a 9 átomos en el anillo, que contiene 1, 2 ó 3 heteroátomos elegido entre N, O y S, donde los demás átomos del anillo son carbonos y donde bicíclico indica que está formado por dos ciclos que tienen en común dos átomos del anillo,
- 25 viii) heterociclilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^8 , donde el heterociclilo se define como un sistema cíclico monovalente saturado o parcialmente insaturado, de 4 a 9 átomos en el anillo, que contiene 1, 2 ó 3 heteroátomos elegido entre N, O y S, donde los demás átomos del anillo son carbonos y donde bicíclico indica que está formado por dos ciclos que tienen en común dos átomos del anillo,
- 30 ix) alquinilo C_{2-6} ;
- 35 x) alquilo C_{1-6} y

xi) alquilo C₁₋₆ sustituido por 1-3 sustituyentes elegidos individualmente entre R⁹;

R¹ se elige entre el grupo formado por:

- i) hidrógeno,
- ii) halógeno y
- 5 iii) alquilo C₁₋₆;

R² se elige entre el grupo formado por:

- i) hidrógeno,
- ii) alquilo C₁₋₆ y
- iii) halogeno-alquilo C₁₋₃,

10 R³ se elige entre el grupo formado por:

- i) hidrógeno,
- ii) halógeno y
- iii) alquilo C₁₋₆,

R⁴ se elige entre el grupo formado por:

- 15 i) hidrógeno,
- ii) halógeno y
- iii) alquilo C₁₋₆ y

R⁵ se elige entre el grupo formado por:

- i) hidrógeno y
- 20 ii) alquilo C₁₋₆,

R⁶ se elige entre el grupo formado por:

- i) hidrógeno y
- ii) alquilo C₁₋₆,

25 R⁷ se elige entre el grupo formado por:

- i) hidrógeno y
- ii) alquilo C₁₋₆,

R⁸ se elige entre el grupo formado por:

- i) halógeno,
- 30 ii) ciano,
- iii) alquilo C₁₋₆,
- iv) halogeno-alquilo C₁₋₆,
- v) alcoxi C₁₋₆,
- vi) halogeno-alcoxi C₁₋₆,
- 35 vii) arilo, el cual se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,

viii) halogeno-arilo, donde el arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo, y

ix) cicloalquilo C_{3-6} ; y

5 R^9 se elige entre el grupo formado por:

i) halógeno,

ii) ciano,

iii) alcoxí C_{1-6} y

iv) halogeno-alcoxí C_{1-6} ,

10 o sus sales farmacéuticamente aceptables.

2. El compuesto según la reivindicación 1, en el que:

X se elige entre el grupo formado por:

15 i) arilo, el cual se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,

ii) arilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^1 , donde el arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,

20 iii) heteroarilo, el cual se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático, y

25 iv) heteroarilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^1 , donde el heteroarilo se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático;

30 Y se elige entre el grupo formado por:

i) $-NHCH_2-$,

ii) $-NH-$, o

iii) está ausente;

Z se elige entre el grupo formado por:

35 i) arilo, el cual se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,

- 15
- 5
- 10
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- ii) arilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^8 , donde el arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,
 - iii) heteroarilo, el cual se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático,
 - iv) heteroarilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^8 , donde el heteroarilo se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático,
 - v) cicloalquilo C_{3-6} ,
 - vi) cicloalquilo C_{3-6} sustituido por 1-2 sustituyentes elegidos individualmente entre R^8 ,
 - vii) heterociclilo, el cual se define como un sistema cíclico monovalente saturado o parcialmente insaturado, de 4 a 9 átomos en el anillo, que contiene 1, 2 ó 3 heteroátomos elegido entre N, O y S, donde los demás átomos del anillo son carbonos y donde bicíclico indica que está formado por dos ciclos que tienen en común dos átomos del anillo,
 - viii) heterociclilo sustituido por 1-2 sustituyentes elegidos individualmente entre R^8 , donde el heterociclilo se define como un sistema cíclico monovalente saturado o parcialmente insaturado, de 4 a 9 átomos en el anillo, que contiene 1, 2 ó 3 heteroátomos elegido entre N, O y S, donde los demás átomos del anillo son carbonos y donde bicíclico indica que está formado por dos ciclos que tienen en común dos átomos del anillo, y
 - ix) alquinilo C_{2-6} ;
- R^1 se elige entre el grupo formado por:
- i) hidrógeno,
 - ii) halógeno y
 - iii) alquilo C_{1-6} ;
- R^2 se elige entre el grupo formado por:
- i) hidrógeno,
 - ii) alquilo C_{1-6} y

iii) halogeno-alquilo C_{1-3} ,

R^3 se elige entre el grupo formado por:

i) hidrógeno,

ii) halógeno y

5 iii) alquilo C_{1-6} ,

R^4 se elige entre el grupo formado por:

i) hidrógeno,

ii) halógeno y

iii) alquilo C_{1-6} y

10 R^5 se elige entre el grupo formado por:

i) hidrógeno y

ii) alquilo C_{1-6} ,

R^6 se elige entre el grupo formado por:

i) hidrógeno y

15 ii) alquilo C_{1-6} ,

R^7 se elige entre el grupo formado por:

i) hidrógeno y

ii) alquilo C_{1-6} y

R^8 se elige entre el grupo formado por:

20 i) halógeno,

ii) ciano,

iii) alquilo C_{1-6} ,

iv) halogeno-alquilo C_{1-6} ,

v) alcoxi C_{1-6} ,

25 vi) halogeno-alcoxi C_{1-6} ,

vii) arilo, el cual se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo,

viii) halogeno-arilo, donde el arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo, y

30 ix) cicloalquilo C_{3-6} ;

o sus sales farmacéuticamente aceptables.

3. El compuesto según una cualquiera de las reivindicaciones 1 o 2, en el que R^2 es

35 metilo, R^3 es F, R^4 es H, metilo o F, R^5 es H y R^6 es H.

4. El compuesto según una cualquiera de las reivindicaciones 1 a 3, en el que X es arilo sustituido por halógeno, donde el arilo se define como un sistema de anillo mono- o bicíclico carbocíclico aromático monovalente, que tiene de 6 a 10 átomos de carbono en el anillo.

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5. El compuesto según una cualquiera de las reivindicaciones 1 a 4, en el que Z es heteroarilo sustituido por 1 o 2 sustituyentes elegidos individualmente entre R⁸ o cicloalquilo C₃₋₆, donde el heteroarilo se define como un grupo carbocíclico aromático que tiene un solo anillo de 4 a 8 eslabones, o múltiples anillos condensados que totalizan de 6 a 14 átomos, en particular de 6 a 10 átomos en el anillo y contienen 1, 2 ó 3 heteroátomos elegidos individualmente entre N, O y S, en particular N y O, y en donde por lo menos un anillo heterocíclico es aromático.

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6. El compuesto según una cualquiera de las reivindicaciones 1 a 5, en el que R⁸ se elige entre el grupo formado por:

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- i) halógeno,
- ii) ciano,
- iii) alquilo C₁₋₆ y
- iv) halogeno-alquilo C₁₋₆.

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7. Un compuesto según una cualquiera de las reivindicaciones 1 a 6, elegido entre el grupo formado por:

(S)-4-{5-[(4-cloro-1-difluormetil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

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(4R,5R)-5-fluor-4-[2-fluor-5-(2-metoxi-fenilamino)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(4R,5R)-4-[5-(2-difluormetoxi-fenilamino)-2-fluor-fenil]-5-fluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

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(4R,5R)-5-fluor-4-{2-fluor-5-[(tiofen-2-ilmetil)-amino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(4R,5R)-5-fluor-4-{2-fluor-5-[(tiofen-3-ilmetil)-amino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(4R,5R)-5-fluor-4-[2-fluor-5-(2-fluor-bencilamino)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

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- (4R,5R)-4-[5-(ciclopropilmetil-amino)-2-fluor-fenil]-5-fluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (4R,5R)-5-fluor-4-{2-fluor-5-[1-(4-fluor-fenil)-1H-pirazol-4-il]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 5 (4R,5R)-5-fluor-4-[2-fluor-5-(4-metil-pirazol-1-il)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (4R,5R)-4-{5-[(4-cloro-1-difluormetil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-5-fluor-4,5-dimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (4R,5R)-4-{5-[(4-cloro-1-metil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-5-fluor-10 4,5-dimetil-5,6-dihidro-4H-[1,3]-oxazin-2-ilamina,
- (4R,5R)-5-fluor-4-{2-fluor-5-[1-(2-metil-5-trifluormetil-oxazol-4-il)-etilamino]-fenil}-4,5-dimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-{5-[(4-cloro-1-difluormetil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 15 (R)-4-{5-[1-(4-cloro-1-metil-1H-pirazol-3-il)-etilamino]-2-fluor-fenil}-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-{5-[1-(5-ciclopropil-isoxazol-3-il)-etilamino]-2-fluor-fenil}-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 20 4-[5-(5-cloro-piridin-3-il)-2-fluor-fenil]-4-difluormetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-[5-(5-cloro-piridin-3-il)-2-fluor-fenil]-4-difluor-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (S)-4-[5-(5-cloro-piridin-3-il)-2-fluor-fenil]-4-difluor-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 25 2-[3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenilamino]-benzonitrilo,
- (R)-5,5-difluor-4-(2-fluor-5-o-tolilamino-fenil)-4,6,6-tri-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-(5-ciclopentilamino-2-fluor-fenil)-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 30 (R)-4-(3',5'-dicloro-4-fluor-bifenil-3-il)-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 3'-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-6-cloro-4'-fluor-bifenil-3-carbonitrilo,
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- (R)-4-[5-(1,1-dioxo-2,3-dihidro-1H-1λ⁶-benzo[b]tiofen-3-il-amino)-2-fluor-fenil]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
5-[3-((4R,5R)-2-amino-5-fluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-nicotinonitrilo,
- 5 7-[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenilamino]-6,7-dihidro-5H-[1]piridina-3-carbonitrilo,
[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-
(3-cloro-6,7-dihidro-5H-[1]-piridin-7-il)-amina,
- 10 8-[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenilamino]-5,6,7,8-tetrahidro-quinolina-3-carbonitrilo,
3-[4-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-pirazol-1-il]-benzonitrilo,
- (R)-4-(5-{1-[4-cloro-1-(2,2-difluor-etil)-1H-pirazol-3-il]-etilamino}-2-fluor-fenil)-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 15 (4R,5R)-4-[5-(3,5-difluor-bencilamino)-2-fluor-fenil]-5-fluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
(R)-4-[3-(5-cloro-piridin-2-il)-7-fluor-3,4-dihidro-2H-benzo-[1,4]oxazin-6-il]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-2-ilamina,
- (R)-4-[5-(6-cloro-benzooxazol-2-il)-2,4-difluor-fenil]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 20 (R)-5,5-difluor-4-{2-fluor-5-[1-(5-metil-2H-pirazol-3-il)-etilamino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
(R)-5,5-difluor-4-{2-fluor-5-[1-(2-metil-5-trifluormetil-oxazol-4-il)-etilamino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]-oxazin-2-ilamina,
- 25 (R)-5,5-difluor-4-{2-fluor-5-[(3-metil-oxetan-3-ilmetil)-amino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
(R)-4-[5-(ciclopropilmetil-amino)-2-fluor-fenil]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-5,5-difluor-4-[2-fluor-5-(tetrahidro-furan-3-ilamino)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 30 (R)-5,5-difluor-4-[2-fluor-5-(oxetan-3-ilamino)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
(R)-5,5-difluor-4-{2-fluor-5-[(tetrahidro-furan-3-ilmetil)-amino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 35 (R)-5,5-difluor-4-{2-fluor-5-[1-(4-fluor-fenil)-1H-pirazol-4-il]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(R)-4-[5-(5-cloro-piridin-3-il)-2,4-difluor-fenil]-5,5-di-fluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

5 (R)-5,5-difluor-4-[2-fluor-5-(2-metoxi-fenilamino)-fenil]-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(R)-5,5-difluor-4-[2-fluor-5-(2-trifluormetil-fenilamino)-fenil]-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(R)-5,5-difluor-4-[2-fluor-5-(6-trifluormetil-piridin-2-il-amino)-fenil]-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

10 (R)-5,5-difluor-4-[2-fluor-5-(3-fenil-ciclopentilamino)-fenil]-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(R)-4-[5-(5-cloro-piridin-3-il)-2-fluor-fenil]-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

15 (R)-4-[1-(3-etinil-fenil)-1H-pirazol-4-il]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(R)-4-(2,4-difluor-5-pirimidin-5-il-fenil)-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(R)-4-[5-(2-cloro-fenilamino)-2-fluor-fenil]-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina y

20 (S)-4-(5-ciclopentilamino-2-fluor-fenil)-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina;

o una sal farmacéuticamente aceptable de los mismos.

8. Un compuesto según una cualquiera de las reivindicaciones 1-7, elegido entre el grupo formado por:

(S)-4-{5-[(4-cloro-1-difluormetil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(4R,5R)-4-[5-(2-difluormetoxi-fenilamino)-2-fluor-fenil]-5-fluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

30 (4R,5R)-4-[5-(ciclopropilmetil-amino)-2-fluor-fenil]-5-fluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(4R,5R)-4-{5-[(4-cloro-1-difluormetil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-5-fluor-4,5-dimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

35 (4R,5R)-4-{5-[(4-cloro-1-metil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-5-fluor-4,5-dimetil-5,6-dihidro-4H-[1,3]-oxazin-2-ilamina,

- (4R,5R)-5-fluor-4-[2-fluor-5-(2-fluor-bencilamino)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (4R,5R)-5-fluor-4-[2-fluor-5-(2-metoxi-fenilamino)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 5 (4R,5R)-5-fluor-4-[2-fluor-5-(4-metil-pirazol-1-il)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (4R,5R)-5-fluor-4-{2-fluor-5-[(tiofen-2-ilmetil)-amino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (4R,5R)-5-fluor-4-{2-fluor-5-[(tiofen-3-ilmetil)-amino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 10 (4R,5R)-5-fluor-4-{2-fluor-5-[1-(2-metil-5-trifluormetil-oxazol-4-il)-etilamino]-fenil}-4,5-dimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (4R,5R)-5-fluor-4-{2-fluor-5-[1-(4-fluor-fenil)-1H-pirazol-4-il]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 15 (R)-4-(2,4-difluor-5-pirimidin-5-il-fenil)-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-(3',5'-dicloro-4-fluor-bifenil-3-il)-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-(5-{1-[4-cloro-1-(2,2-difluor-etil)-1H-pirazol-3-il]-etilamino}-2-fluor-fenil)-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 20 (R)-4-(5-ciclopentilamino-2-fluor-fenil)-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-[3-(5-cloro-piridin-2-il)-7-fluor-3,4-dihidro-2H-benzo-[1,4]oxazin-6-il]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-2-ilamina,
- 25 (R)-4-[5-(1,1-dioxo-2,3-dihidro-1H-1λ6-benzo[b]tiofen-3-il-amino)-2-fluor-fenil]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-[5-(2-cloro-fenilamino)-2-fluor-fenil]-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-[5-(5-cloro-piridin-3-il)-2,4-difluor-fenil]-5,5-di-fluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 30 (R)-4-[5-(5-cloro-piridin-3-il)-2-fluor-fenil]-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 35 (R)-4-[5-(6-cloro-benzooxazol-2-il)-2,4-difluor-fenil]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

- (R)-4-[5-(ciclopropilmetil-amino)-2-fluor-fenil]-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-{5-[(4-cloro-1-difluormetil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 5 (R)-4-{5-[1-(4-cloro-1-metil-1H-pirazol-3-il)-etilamino]-2-fluor-fenil}-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-4-{5-[1-(5-ciclopropil-isoxazol-3-il)-etilamino]-2-fluor-fenil}-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-5,5-difluor-4-(2-fluor-5-o-tolilamino-fenil)-4,6,6-tri-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 10 (R)-5,5-difluor-4-[2-fluor-5-(2-metoxi-fenilamino)-fenil]-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-5,5-difluor-4-[2-fluor-5-(2-trifluormetil-fenilamino)-fenil]-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-5,5-difluor-4-[2-fluor-5-(3-fenil-ciclopentilamino)-fenil]-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 15 (R)-5,5-difluor-4-[2-fluor-5-(6-trifluormetil-piridin-2-il-amino)-fenil]-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-5,5-difluor-4-[2-fluor-5-(oxetan-3-ilamino)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 20 (R)-5,5-difluor-4-[2-fluor-5-(tetrahidro-furan-3-ilamino)-fenil]-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-5,5-difluor-4-{2-fluor-5-[(3-metil-oxetan-3-ilmetil)-amino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-5,5-difluor-4-{2-fluor-5-[(tetrahidro-furan-3-ilmetil)-amino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 25 (R)-5,5-difluor-4-{2-fluor-5-[1-(2-metil-5-trifluormetil-oxazol-4-il)-etilamino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (R)-5,5-difluor-4-{2-fluor-5-[1-(4-fluor-fenil)-1H-pirazol-4-il]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 30 (R)-5,5-difluor-4-{2-fluor-5-[1-(5-metil-2H-pirazol-3-il)-etilamino]-fenil}-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- (S)-4-(5-ciclopentilamino-2-fluor-fenil)-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,
- 35 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-(3-cloro-6,7-dihidro-5H-[1]-piridin-7-il)-amina,

2-[3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenilamino]-benzonitrilo,

3'-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-6-cloro-4'-fluor-bifenil-3-carbonitrilo,

5 5-[3-((4R,5R)-2-amino-5-fluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-nicotinonitrilo,

7-[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenilamino]-6,7-dihidro-5H-[1]piridina-3-carbonitrilo y

10 8-[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenilamino]-5,6,7,8-tetrahidro-quinolina-3-carbonitrilo,

o una sal farmacéuticamente aceptable de los mismos.

9. Un compuesto según una cualquiera de las reivindicaciones de 1 a 8, elegido entre el grupo formado por:

15 (4R,5R)-4-[5-(ciclopropilmetil-amino)-2-fluor-fenil]-5-fluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

(4R,5R)-4-{5-[(4-cloro-1-difluormetil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-5-fluor-4,5-dimetil-5,6-dihidro-4H-[1,3]oxazin-2-ilamina,

20 (4R,5R)-4-{5-[(4-cloro-1-metil-1H-pirazol-3-ilmetil)-amino]-2-fluor-fenil}-5-fluor-4,5-dimetil-5,6-dihidro-4H-[1,3]-oxazin-2-ilamina,

[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]- (3-cloro-6,7-dihidro-5H-[1]-piridin-7-il)-amina,

5-[3-((4R,5R)-2-amino-5-fluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-nicotinonitrilo y

25 7-[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenilamino]-6,7-dihidro-5H-[1]piridina-3-carbonitrilo.

10. Una composición farmacéutica que contiene un compuesto de la fórmula I según una cualquiera de las reivindicaciones 1-9 y un vehículo (excipiente) farmacéuticamente
30 aceptable y/o una sustancia auxiliar farmacéuticamente aceptable.

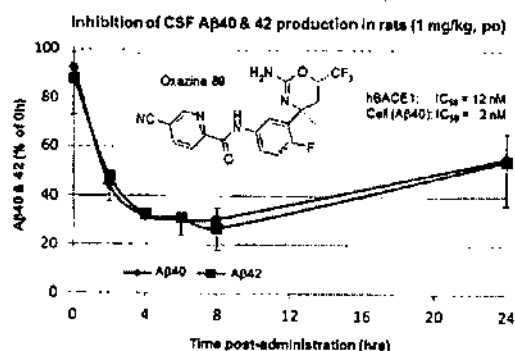
β -Secretase (BACE1) Inhibitors with High in Vivo Efficacy Suitable for Clinical Evaluation in Alzheimer's Disease

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Supporting Information

ABSTRACT: An extensive fluorine scan of 1,3-oxazines revealed the power of fluorine(s) to lower the pK_a and thereby dramatically change the pharmacological profile of this class of BACE1 inhibitors. The CF₃-substituted oxazine 89, a potent and highly brain penetrant BACE1 inhibitor, was able to reduce significantly CSF A β 40 and 42 in rats at oral doses as low as 1 mg/kg. The effect was long lasting, showing a significant reduction of A β 40 and 42 even after 24 h. In contrast to 89, compound 1b lacking the CF₃ group was virtually inactive in vivo.



INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the major cause of dementia in the elderly. The clinical symptoms of AD are progressive memory loss, learning impairment, and behavioral and psychiatric disturbances. The predominant pathological hallmarks of the disease are (1) amyloid plaques, i.e., extracellular deposits of polymerized amyloid- β peptides (A β), and (2) intracellular aggregates of mis-folded tau protein. Formation of these pathologies can precede the clinical disease symptoms by years. The final disease pathology is further characterized by pronounced neuroinflammation and widespread neuronal loss. As no causative treatment is currently available, there is an urgent need for new, efficacious therapies.¹ Amyloid aggregates are considered to be the earliest pathology and, according to the amyloid cascade hypothesis, the ultimate cause of the disease.^{2–5} Their main constituents, the amyloid- β peptides, are derived from the β -amyloid precursor protein (APP) via proteolytic processing by β -secretase (BACE1) and γ -secretase. Both APP and BACE1 are type-1 membrane proteins highly expressed in the brain. BACE1 is an aspartic protease of 501 amino acids length, organized into an extracellular domain with signal peptide, prodomain and catalytic domain, a trans-membrane domain and a short cytoplasmic stretch of 22 amino acids. Its principal cleavage site in the APP extracellular domain forms the N-terminus of A β (position D672 of the longest APP isotype); a secondary cleavage site is localized at

position E682. Processing of APP occurs predominantly in endosomal vesicles, in agreement with the "acidic pH" optimum of the enzyme.^{6–8} The unique and essential role of BACE1 for the generation of A β has been convincingly shown in mice where both alleles have been ablated by genetic means. These mice do not form A β , nor do they cleave APP at the known BACE1-positions.^{9,10} It has furthermore been demonstrated that upon crossing BACE1 knockout mice and APP-transgenic mice, the formation of brain amyloid is inhibited. Interestingly, ablation of only one BACE1 allele was enough to reduce amyloidosis significantly. These data suggest that pharmacological inhibition of BACE1 should also reduce amyloidosis and that partial inhibition should be sufficient to achieve therapeutic efficacy.^{11,12} Taken together, this makes BACE1 a prime target for anti-amyloid therapy. Partial inhibition of BACE1 may also be the preferred therapeutic target because a number of additional BACE1 substrates have been described. Of special interest is neuregulin 1 (NRG1) processing because close examination of BACE1 knockout mice has revealed that in these animals the myelination of peripheral and possibly central nerves is severely reduced, presumably due to the absence of NRG1 processing.^{13,14} Importantly, this adverse effect was not observed in animals that retained one functional allele.

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The aspartyl protease BACE2 is a close homologue of BACE1. BACE2, however, cleaves APP preferentially at a position downstream of the BACE1 cleavage sites at F691 of APP, which does not produce amyloidogenic A β peptides. BACE2 is primarily expressed in peripheral tissue, in particular in pancreatic islets.¹⁵ Its physiological function was poorly understood for some time,⁸ but recent evidence suggests that it may play a role in glucose homeostasis.¹⁵

Soon after the discovery of BACE1 in 1999,^{16–20} the enzyme became a prime target of both academic and industrial research. Initial inhibitor design focused on statine type structures engaging with the two aspartic acids in the catalytic site. While potent in vitro, the peptidic nature of these inhibitors rendered them poorly brain penetrant. To overcome this shortcoming, less peptidic hydroxyethylamine (HEA) isosters subsequently became the focus of research. Good brain penetration, however, remained the exception rather than the rule. This has been ascribed to the flexibility of HEAs and their high number of hydrogen bond donors and acceptors.²¹

Our research focused on cyclic, conformationally rigid structures that interact with the two aspartic acids of BACE1. An HTS campaign yielded a small fragment of a weakly active (IC₅₀ = 41.2 μ M) 2-amino-4H-5,6-dihydro-[1,3]thiazine derivative A (Figure 1) which could be cocrystallized with

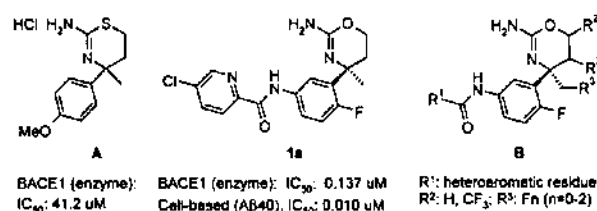


Figure 1. Lead compound 1a and targeted fluoro-oxazines B.

BACE1, offering unique opportunities for optimization. This heterocycle was first synthesized at Roche more than 30 years ago in a project looking for novel analgesics²² and has later

been explored by various research groups^{23–28} as a headgroup interacting with the aspartic acids of BACE1.

Previously we described the identification and characterization of a number of cyclic amidines that were devoid of substantial in vivo activity due to low penetration into the CNS.²⁹ In this paper we disclose our efforts to convert our initial lead compound, the 2-amino-4H-5,6-dihydro-[1,3]-oxazine derivative 1a into fluorine containing oxazines B, a compound class characterized by high in vivo activity and good overall properties.

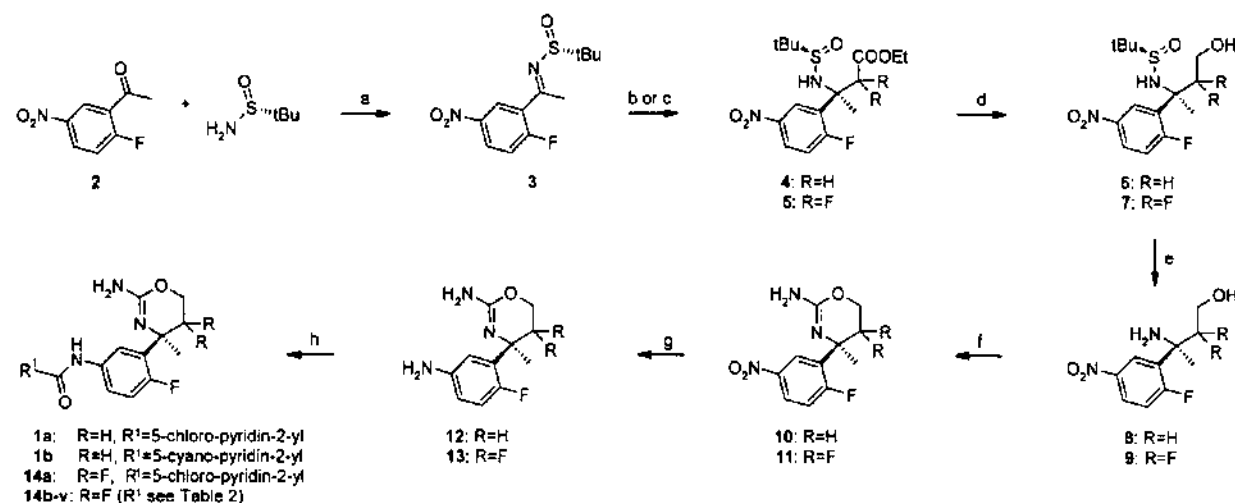
CHEMISTRY

The syntheses of oxazines 1a–b and difluoro-oxazines 14a–v (Scheme 1) started with elegant work developed by Ellman.³⁰ Condensation of the chiral auxiliary (R)-tert-butanesulfinamide and acetophenone 2 in the presence of Ti(OEt)₄ gave the sulfinyl imine 3. Transmetalation of the lithium enolate of ethyl acetate using TiCl(OiPr)₃ generated the titanium enolate, which reacted with the sulfinyl imine 3 to furnish the ester 4 as a single diastereoisomer. Alternatively, the Reformatsky reaction of the sulfinyl imine 3 and ethyl bromodifluoroacetate³¹ furnished the α,α -difluoroester 5.

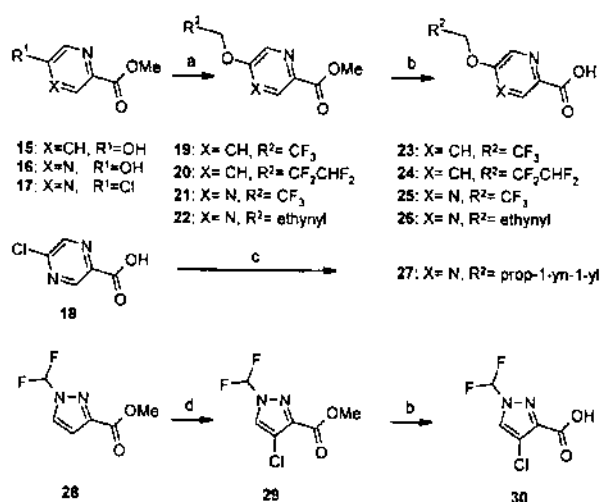
The ester group in 4 and 5 was then reduced to the alcohols 6 and 7 with LiBH₄, which was followed by cleavage of the chiral auxiliary under acidic conditions to give the amino alcohols 8 and 9.³² Cyclization of the amino alcohols 8 and 9 was readily accomplished with cyanogen bromide to give the oxazines 10 and 11, which were reduced to the corresponding anilines 12³³ and 13 by catalytic hydrogenation. Amide formation was accomplished without prior protection of the amidine moiety using 4-(4,6-dimethoxy[1,3.5]triazin-2-yl)-4-methylmorpholinium chloride hydrate,³⁴ furnishing the target compounds 1a–b and 14a–v (Table 2). The absolute configurations were assigned based on X-ray structures of 1b and 14d complexed to BACE1.

The commercially unavailable acids 23–26 (Scheme 2), required to introduce R¹ residues in difluoro-oxazine 14 (Table 2), were made available from pyridine and pyrazine derivatives

Scheme 1. Synthesis of Oxazines 1a–b and Difluoro-oxazines 14a–v^a



^aReagents and conditions: (a) Ti(OEt)₄, THF, 50–70 °C. (b) 3→4: MeCOOEt, LDA, TiCl(OiPr)₃, THF, –78 °C. (c) 3→5: BrCF₂COOEt, Zn, CuCl, THF, 35 °C. (d) LiBH₄, THF, 0 °C. (e) HCl/1,4-dioxane/MeOH, 22 °C. (f) BrCN, EtOH, 85 °C. (g) H₂, Pd/C, EtOH, 22 °C. (h) 4-(4,6-Dimethoxy[1,3.5]triazin-2-yl)-4-methylmorpholinium chloride hydrate (DMTMM), MeOH, 22 °C.

Scheme 2. Synthesis of Commercially Unavailable Building Blocks 23–27 and 30^a

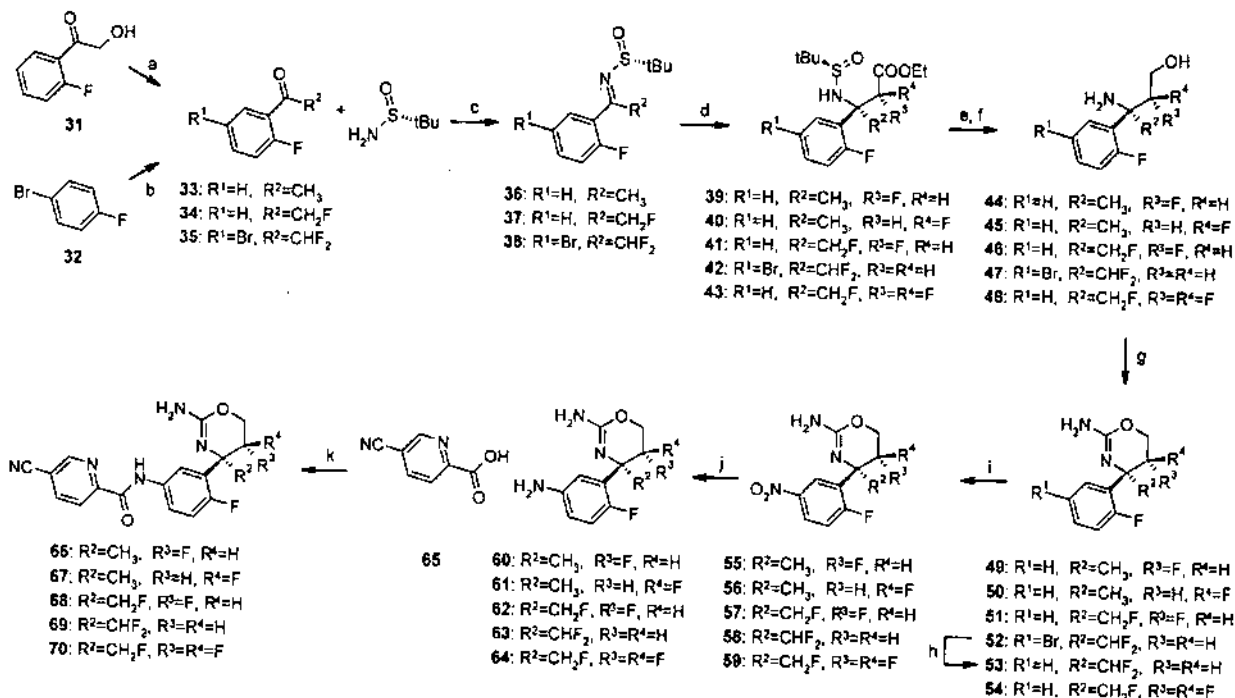
^aReagents and conditions: (a) 15→19: CF₃CH₂OTf, NaH, DMF, 22 °C. 15→20: CHF₂CH₂OTf, K₂CO₃, acetone. 17→21: CF₃CH₂OH, Cs₂CO₃, DMF. 17→22: prop-2-yn-1-ol, *tert*-BuOK, 1,4-dioxane, 22 °C. (b) LiOH or NaOH, THF/H₂O, 22 °C to reflux. (c) But-2-yn-1-ol, *tert*-BuOK, DMF, 65 °C. (d) N-chlorosuccinimide, DMF, 50 °C.

15 and 17 via the alkylated esters 19–22. Attempted alkylation of the hydroxy-pyrazine ester 16 with 3-bromopropyne did not lead to the expected *O*-alkylated product 22 but furnished the *N*-alkylated product (5-oxo-4-prop-2-ynyl-4,5-dihydro-pyrazine-2-carboxylic acid methyl ester, structure not shown).

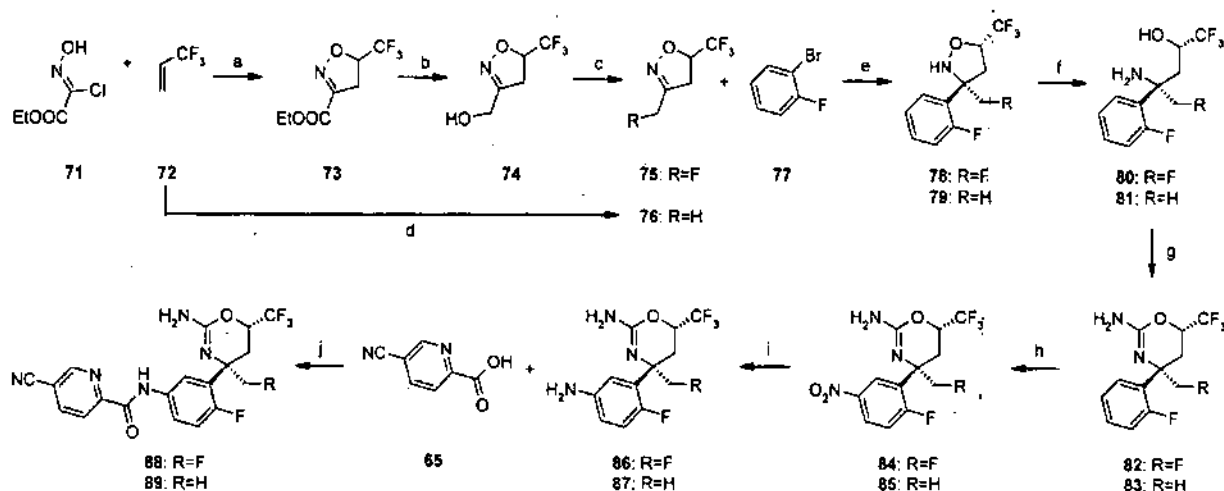
Compound 27 was prepared directly from the acid 18 without proceeding via the corresponding ester. Finally, chlorination of the pyrazole ester 28 with *N*-chlorosuccinimide followed by saponification delivered the chloro-pyrazole derivative 30.

To access fluoro-oxazines 66–70 (Scheme 3) having fluorines attached at positions R²–R⁴, the synthetic strategy was modified. Occasionally the alcohol group in 6–9 (Scheme 1) can undergo an intramolecular aromatic substitution of the fluorine, which is activated by the nitro group, to give the corresponding nitro-chromane derivatives (structures not shown). We therefore introduced the nitro group at a later stage of the synthesis. The commercially unavailable ketones 34 and 35 (Scheme 3) were made available by fluorination of the alcohol 31 or by acylation of 32 using ethyl disfluoroacetate. Sulfinyl imines 36–38, prepared as described in Scheme 1, were then subjected to the Reformatsky reaction to afford the esters 39–43. The reaction of 36 with BrCHFCOOEt yielded a separable 4:1-mixture of the epimers 39 and 40. Conversion of 37 with BrCHFCOOEt gave a separable 8:1-mixture of 41 as the major isomer and the corresponding epimer (R³ = H, R⁴ = F). Oxazines 49–51 and 53–54, prepared in analogy to Scheme 1, were then nitrated with fuming nitric acid, followed by reduction of the nitro group and amide formation in the usual manner, to give the targeted fluorinated oxazines 66–70. The relative and absolute configurations were assigned based on X-ray structures of 66–70 complexed to BACE1.

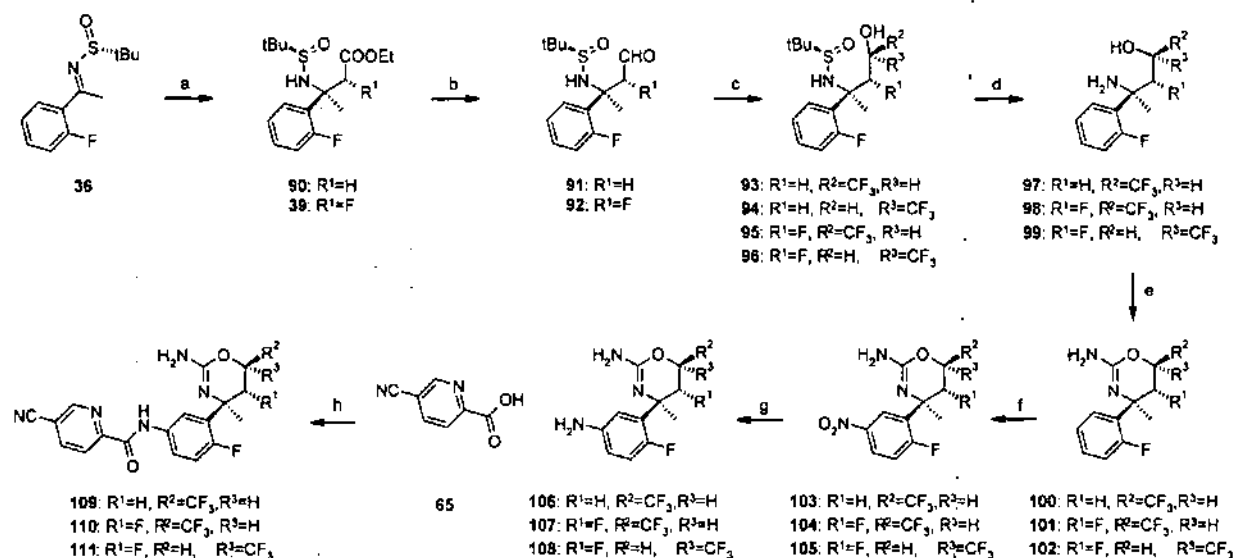
The synthesis of trifluoromethyl substituted oxazines 88 and 89 (Scheme 4) required a different strategy. Ready access to CF₃ substituted 3-amino-propan-1-ol derivatives 80 and 81 is achieved using a dipolar cycloaddition reaction of a nitrile oxide

Scheme 3. Synthesis of Fluoro-oxazines 66–70^a

^aReagents and conditions: (a) 31→34: nonafluoro-*n*-butansulfonyl fluoride, triethylamine trihydrofluoride, CH₂Cl₂, 0 to 22 °C. (b) 32→35: LDA, F₂CHCOOEt, THF, –70 to –10 °C. (c) Ti(OEt)₄, THF, 70 °C. (d) 39, 40, and 41: BrCHFCOOEt, 42: BrCH₂COOEt, 43: BrCF₂COOEt, Zn, CuCl, THF. (e) LiBH₄, EtOH, 22 °C. (f) aq HCl, THF, 22 °C. (g) BrCN, *i*-PrOH, or EtOH, 80 °C. (h) H₂, Pd/C, NaOAc, MeOH, 22 °C. (i) HNO₃, H₂SO₄, TFA, 0 °C. (j) H₂, Pd/C, Et₃N, EtOH, 22 °C. (k) DMTMM, MeOH, 0–22 °C.

Scheme 4. Synthesis of Trifluoromethyl-oxazines 88 and 89^a

^aReagents and conditions: (a) NaHCO₃, EtOAc, -65 to 22 °C. (b) NaBH₄, EtOH, 0 °C. (c) Deoxo-Fluor, CH₂Cl₂, -78 to 22 °C. (d) 72→76: nitroethane, phenylisocyanate, Et₃N (cat.), Et₂O, -78 to 22 °C. (e) THF, *n*-BuLi, BF₃·Et₂O, -78 to 22 °C. (f) HCOONH₄, Pd/C, EtOH, 22 °C. (g) BrCN, EtOH, 85 °C. (h) H₂SO₄, HNO₃, 0 °C. (i) H₂, Pd/C, EtOH, Et₃N, 22 °C. (j) DMTMM, MeOH, 0 to 22 °C.

Scheme 5. Synthesis of Fluoro-oxazines 109–111^a

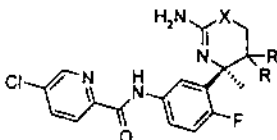
^aReagents and conditions: (a) 36→39: BrCH₂COOEt, Zn, CuCl, THF. 36→90: BrCH₂COOEt, Zn, CuCl, THF. (b) DIBAL, toluene, -76 °C. (c) Trimethyl(trifluoromethyl)silane, THF, TBAF, 0 °C. (d) aq HCl, THF, 22 °C. (e) BrCN, EtOH, 85 °C. (f) H₂SO₄, HNO₃, 0 °C. (g) H₂, Pd/C, EtOH, Et₃N, 22 °C. (h) DMTMM, MeOH, 0–22 °C.

and an olefin³⁵ as the key step. Utilizing carefully optimized conditions, the nitrile oxide was slowly generated in situ from 2-chloro-2-hydroxy-imino ester 71 in a heterogeneous mixture of NaHCO₃ in ethyl acetate in the presence of 3,3,3-trifluoropropene (72) to give the 4,5-dihydro-isoxazole ester 73 in quantitative yield. Reduction of the ester 73 followed by fluorination of the alcohol 74 with Deoxo-Fluor furnished the desired fluoro compound 75. 3-Methyl-4,5-dihydro-isoxazole derivative 76 was directly accessible by reacting nitroethane and phenyl isocyanate to generate the nitrile oxide in situ in the presence of 72. Arylation of the imines 75 and 76 with 1-bromo-2-fluorobenzene (77) and *n*-BuLi occurred from the sterically less hindered side to give the racemic isoxazolidines 78 and 79, which were separated by chiral HPLC, affording the

chiral compounds 78 and 79. Transfer hydrogenation employing ammonium formate and Pd/C led to the amino alcohols 80 and 81, which were then converted to the CF₃-substituted oxazines 88 and 89 following the procedures employed in Scheme 3. The relative and absolute configurations were assigned based on X-ray structures of 88 and 89 complexed to BACE1.

The last set of CF₃-substituted oxazines 109–111 (Scheme 5), not accessible by methods hitherto described, again required a different synthetic strategy. Esters 90 and 39 were reduced with DIBAL at -76 °C to give the aldehydes 91 and 92, which were reacted with trimethyl(trifluoromethyl)silane (Ruppert–Prakash reagent), yielding the alcohols 93–96. The reaction of 91 afforded a separable 1:2-mixture of epimers 93 and 94, and

Table 1. In Vitro and in Vivo Profiles of Oxazines 1a and 14a and Thiazines 112 and 113



compd	X	R	pK_a^b	IC_{50} (μM) ^a			shift BACE1 enzyme vs cell-based	brain A β 40 reduction in mice at 30 mg/kg ^d (%)
				hBACE1 enzyme	hBACE2 enzyme	A β 40 cell-based ^c		
1a	O	H	9.8	0.137	0.052	0.010	14	29
14a	O	F	6.3	0.026	0.015	0.017	2	69
112	S	H	9.0	0.016	0.009	0.001	16	92
113	S	F	6.1	0.014	0.010	0.004	3	0

^a IC_{50} values are means of at least 2 independent experiments. ^b pK_a determined by capillary electrophoresis. ^cHEK293 cells transfected with wild-type human APP. ^dCompound given orally, $n = 3-4$, 4h post dose, mean reduction as compared to vehicle control.

aldehyde 92 gave a separable 2:1-mixture of epimers 95 and 96 with inverse selectivity. Conversion of alcohols 93, 95, and 96 to the target compounds 109–111 followed well-established procedures as described in previous Schemes. The relative and absolute configurations were assigned based on X-ray structures of 109–111 complexed to BACE1.

RESULTS AND DISCUSSION

At the outset of the BACE project, we envisaged both BACE1 and BACE2 as viable targets for AD and type 2 diabetes, respectively. The recently described oxazine 1a^{29,36,37} (for other 1,3-oxazine BACE1 inhibitors see^{26,27,38}) looked particularly suited for the diabetes target, which is primarily expressed in peripheral tissue. Compound 1a showed a high enzymatic inhibitory activity at BACE2 (Table 1, $IC_{50} = 0.052 \mu M$) and was hardly brain penetrant as evident from the low brain/plasma ratio in mice (0.13 at 30 mg/kg), resulting in a marginal activity of 29% reduction of A β 40 at 30 mg/kg in our wild-type mice model (vide infra) we use for the AD indication. An undesired potential liability of 1a, however, was the high basicity ($pK_a = 9.8$), giving rise to potential phospholipidosis³⁹ and polypharmacology.^{40,41} To overcome these liabilities, we introduced fluorines into the headgroup⁴² as depicted in difluoro-oxazine 14a.⁴³ Indeed, this modification reduced the pK_a by 3.5 log units compared to 1a and improved the enzymatic potency at BACE1 and BACE2 5- and 3-fold, respectively. Unexpectedly, however, 14a readily penetrated the brain (brain/plasma ratio in mice = 2.3 at 30 mg/kg) and therefore showed good in vivo activity in mice with a 69% reduction of A β 40 at 30 mg/kg. Obviously, this serendipitously found effect of fluorinated oxazines now rendered this compound class much more appropriate for the AD indication than type 2 diabetes.

For comparison, the thiazine 112²³ and its fluorine congener 113,³² both described by Shionogi, were also prepared. Interestingly, thiazine 112 was very potent in vivo (92% A β 40 reduction at 30 mg/kg), while the oxazine 1a was not. Most surprisingly, the fluorine congener 113, expected to be even more potent than 112, was inactive in vivo. Another remarkable difference was the impact of basicity on the shift between enzymatic and cellular BACE1 potency. Compounds 1a and 112 with high basicity ($pK_a \geq 9$) showed a significantly higher cellular activity (14- and 16-fold, respectively), whereas compounds 14a and 113 with low basicity (<6.5) did not (2

and 3-fold, respectively). The oxazine 14a served as a basis for further optimization of the in vivo activity.

SAR of R¹ Substituents. Next we explored various R¹ aromatic residues all having a nitrogen atom in *ortho* position to the amide group (Table 2) to direct the aromatic residue into the S3 pocket (vide infra).

Out of a number of 6-membered heterocycles explored, pyridines, pyrimidines, and pyrazines showed promising in vitro activities. Pyridines 14a–k having one substituent in *para* position to the amide showed enzymatic inhibition at IC_{50} values below $0.3 \mu M$, most of them with good cellular activity. The nitrile 14d displayed already an improved in vivo activity compared to the chloride 14a with A β 40 reductions of 78 and 28%, respectively, at a dose of 10 mg/kg. In addition, favorable mouse PK data for 14d (Cl = 22 mL/min/kg, $T_{1/2} = 1.8$ h, Vss = 2.9 L/kg, $F = 52\%$) encouraged us to explore further substitution patterns. The *o*- and *p*-disubstituted pyridines 14l and 14m were virtually equipotent to the monosubstituted chloride 14a both in the enzymatic as well as the cell-based assay, but 14m showed a higher in vivo activity compared to 14a (77% vs 28% reduction of A β 40 at 10 mg/kg). The pyrimidine 14n was 3-fold less active in terms of enzymatic potency compared to the pyridine 14a. Pyrazines 14o–14r showed good enzymatic inhibition activity ($IC_{50} = 0.027-0.006 \mu M$), and examples 14o and 14p were clearly more potent in the enzyme/cell-based assays compared to their corresponding pyridine counterparts 14g (2/3-fold) and 14j (4/3-fold), respectively. Interestingly, the propynyloxy-pyrazine 14q was very potent in vivo (93% reduction of A β 40 at 10 mg/kg). Out of a number of 5-membered aromatic heterocycles explored, pyrazoles looked particularly promising. A first example, the methyl pyrazole 14s, was only moderately active in the enzyme/cell-based assays ($IC_{50} = 0.446/0.110 \mu M$) and showed very low metabolic stability in mouse microsomes (Cl = 200 μL /min/mg protein). Potential labile positions, i.e., the methyl group and position 4 of the pyrazole, were therefore blocked by fluorines and a chlorine substituent, respectively. As expected, metabolic stability in mouse microsomes improved considerably in 14t–v (Cl = 98, 59, and 9 μL /min/mg protein, respectively) and higher enzymatic potencies were also observed ($IC_{50} = 0.151, 0.036, \text{ and } 0.019 \mu M$, respectively). Disappointingly, however, the in vivo activity of 14v remained rather modest, with a 57% reduction of A β 40 at 30 mg/kg. The shift between enzyme and cell-based activity was in the majority of examples rather small (6-fold and less, with the exception of a 13-fold shift of the alkyne derivative 14q) and in general in

Table 2. SAR of R1 Substituents and in Vivo Properties of Difluoro-oxazines 14a–v

14a	14b	14c	14d	14e	14f	14g
14h	14i	14j	14k	14l	14m	14n
14o	14p	14q	14r	14s	14t	14u
14v						
IC ₅₀ (μM) ^a		IC ₅₀ (μM) ^a				
compd	hBACE1 enzyme	hBACE2 enzyme	selectivity BACE 2/1	Aβ40 cell-based ^b	shift BACE1 enzyme vs cell-based	brain Aβ40 reduction in mice at 30/10 mg/kg ^c (%)
14a	0.026	0.015	0.6	0.017	2	69/28
14b	0.294	0.036	0.1	0.230	1	
14c	0.066	0.022	0.3	0.013	5	
14d	0.023	0.024	1	0.021	1	84/78
14e	0.087	0.030	0.3	0.087	1	
14f	0.032	0.047	1	0.024	1	27/–
14g	0.051	0.045	1	0.061	1	64/–
14h	0.057	0.245	4	0.054	1	64/–
14i	0.184	0.904	5	0.480	–3	
14j	0.108	7.490	69	0.290	–3	
14k	0.042	9.440	225	0.100	–2	
14l	0.017	0.010	0.6	0.016	1	
14m	0.026	0.012	0.5	0.013	2	91/77
14n	0.067	0.023	0.3	0.023	3	
14o	0.027	0.119	4	0.022	1	95/66
14p	0.025	0.943	38	0.110	–4	
14q	0.013	0.175	13	0.001	13	98/93
14r	0.006	0.101	17	0.001	6	94/28
14s	0.446	0.106	0.2	0.110	4	
14t	0.151	0.031	0.2	0.131	1	
14u	0.036	0.018	0.5	0.017	2	
14v	0.019	0.011	0.6	0.013	1	57/–

^aIC₅₀ values are means of at least two independent experiments. ^bHEK293 cells transfected with wild-type human APP. ^cCompound given orally, *n* = 3–4, 4 h post dose, mean reduction as compared to vehicle control.

favor of higher cellular activity. The (fluoro)alkoxy derivatives 14i–14k and 14p, however, were found to have “negative” shifts (2–4 fold) in favor of higher enzymatic potencies, clearly demonstrating that not only the p*K_a*, but also the nature of R¹ substituents had an impact on the shift.

X-ray Structures and BACE2 Selectivity. The catalytic aspartate residues D93 and D289 form the central recognition motif in the BACE1 binding site (Figure 2). The active site is partially covered by a flexible hairpin loop commonly referred to as the “flap” (Y129–E138).⁴⁴ In apo structures of BACE1 or in complexes with small molecule inhibitors containing an amidine binding motif, the flap backbone is approximately 12 Å away from the side-chains of D93 and D289 (the “open” position). In complexes with peptidomimetic transition state analogues, the flap moves approximately 4 Å closer to the

binding site (the “closed” position).⁴⁴ There is an additional flexible region, the “10s loop”⁴⁴ (G69–G74), forming one side of the S3 pocket. In BACE1, the 10s loop is either in an “up” or “down” conformation,⁴⁵ whereas in BACE2 the 10s loop has been observed in a down conformation by Ostermann et al.⁴⁶ and by ourselves in the complex with 14i (Figure 3). The S3 pocket is much larger in the up conformation, so this difference can be exploited to modulate BACE1/2 selectivity as well as selectivity against other single chain aspartyl proteases.⁴⁴ The binding pocket in S1 and the region at the catalytic aspartates are almost identical between BACE1 and BACE2. Conformational variation around the active site of BACE1 has recently been discussed in detail by Xu et al.;⁴⁷ we will analyze BACE2 flexibility in detail separately.⁴⁸

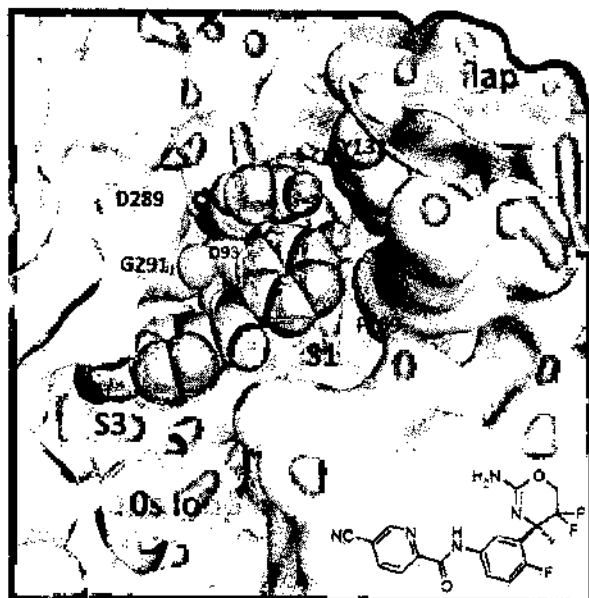


Figure 2. 14d bound to the BACE1 binding site. The amidine recognition motif is tightly bound to the catalytic aspartates D289 and D93 (partially hidden) in the bottom of the pocket. The fluorine atoms in the headgroup and the *o*-F substituent of the phenyl ring (marked in green) form a network of vdW interactions to Y132 and to F169. An additional H-bond is established between the NH of the amide bond of the ligand and the backbone carbonyl oxygen of G291. The *para* nitrile substituent displaces bound water molecules from the S3 pocket.

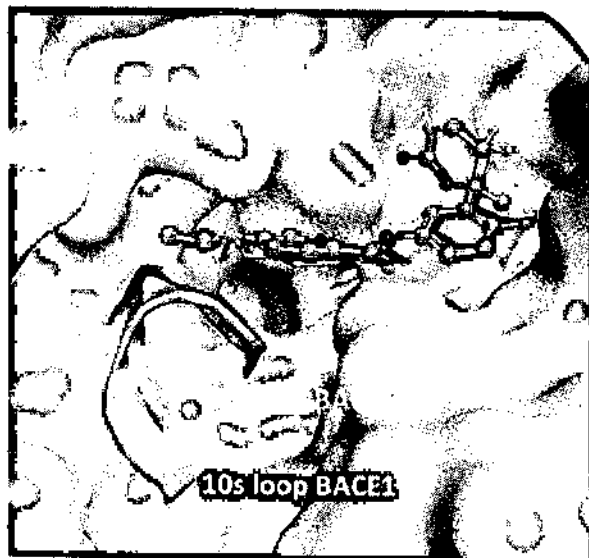


Figure 3. 14i bound to BACE1 (yellow, ball and stick representation) and BACE2 (magenta, stick representation). The molecular surface has been generated for BACE1, and the 10s loops of BACE1 and BACE2 are depicted separately. The ethoxy side chain in 14i prefers a planar orientation around the C–O bond. When bound to BACE2, the corresponding torsion angle deviates significantly from planarity because the S3 pocket in BACE2 is tighter than in BACE1 due to the “down” conformation of the 10s loop in BACE2.

In Figure 2, the topology of the BACE1 active site bound to 14d is highlighted. Tight hydrogen bonds are formed between

the protonated amidine motif in the oxazine headgroup and the catalytic aspartates. The fluorine atoms in position 5 and the *o*-F substituent of the phenyl ring (S1 pocket) are engaged in a network of van der Waals interactions to Y132 located in the flap and to F169 at the rim of the S1 pocket. A hydrogen bond is formed between the NH of the amide bond in 14d and the backbone carbonyl oxygen of G291. The *para* nitrile substituent projects into the S3 pocket and displaces bound water molecules seen in the apo structure, thereby enhancing binding affinity. The proper orientation in the S3 pocket is supported by a stabilization of the conformation of the pyridine ring via a favorable electrostatic interaction between the pyridyl N and the NH of the amide bond in 14d.

The role of the 10s loop in modulating BACE1/2 selectivity can be seen in the ratios of inhibition constants of the compounds 14g–k (Table 2). As is evident in Figure 3, the S3 pocket in BACE2 is smaller than in BACE1 because the 10s loop in BACE2 is observed here in a down conformation. The IC_{50} ratio between BACE2 and BACE1 increases with the growing chain length of the S3 alkyl substituent. Longer chain lengths can only be accommodated in the BACE2 S3 pocket by adopting an unfavorable ligand conformation as depicted in Figure 3. The ethoxy side chain in 14i prefers a planar orientation around the C–O bond. When bound to BACE2, the corresponding torsion angle deviates significantly from planarity, and this conformational strain translates into a reduced inhibition of BACE2. A similar rationale applies for the increase in selectivity with an increasing number of F substituents. To avoid repulsive contacts between F-substituents and carbonyl oxygen atoms of the protein, the tighter S3 pocket in BACE2 requires more strained conformations of the S3 substituent than the more spacious pocket in BACE1.

Fluorine Scan of Oxazines. From various R1 aromatic residues explored, we selected the nitrile 14d for further optimization based on its balanced profile, i.e., good in vitro activities, no shift between enzymatic and cell-based assay, and a favorable in vivo activity. The crucial role of the pK_a on the pharmacological profile as found in the difluoro-oxazine 14a prompted us to investigate extensively the impact of fluorines on various parameters. Oxazines with fluorines appended at different positions in the headgroup (Table 3) were prepared spanning a pK_a range of almost five log units. The head groups in Table 3 are ordered according to decreasing pK_a values. Below we discuss the impact of fluorines on the pK_a , the lipophilicity, the enzymatic potency, the shift of enzyme vs cell-based activity, P-gp efflux, and hERG inhibition, most of these parameters eventually affecting in vivo activity.

The configuration of a fluorine atom attached directly to the oxazine ring has a strong impact on the pK_a . Thus, the “down” configured fluorine in 66 showed a pK_a decrement of 1.6 (ΔpK_a of 1b and 66), whereas the “up” configured fluorine in 67 induced a larger pK_a decrement of 2.3. Similar effects were also observed in fluoro-piperidines.^{49,50} The difluoro oxazine 14d showed exactly the sum of both contributions, i.e., a pK_a decrement of 3.9. The two fluorines in compound 69,^{37,51} also located at the β -position to the ring nitrogen, induced a lower pK_a decrement of 2.4 compared to those of difluoro oxazine 14d. It appears that fluorines with limited conformational flexibility as present in 14d induced a larger pK_a decrement compared to those in 69 having more flexibility. The CF_3 group in compounds 109 and 89 still showed a remarkably large pK_a decrement of 2.4 and 2.7, respectively, despite the longer distances through which the fluorines exert

Table 3. Head Group Fluorine Scan and in Vitro/in Vivo Properties of Fluorinated Oxazines

Chemical structures of compounds 1b, 66, 67, 69, 109, 89, 68, 88, 111, 110, 14d, and 70 are shown. The structures are 2,2,6,6-tetramethyl-1,3-dioxane-4-carbonyl derivatives with various substituents on the dioxane ring.

IC₅₀ (μM)^a

compd	pK _a ^b	ΔpK _a to 1b	log D ^c	log P	Δlog D/log P	hBACE1 enzyme	Aβ40 cell-based ^d	shift BACE1 enzyme vs cell- based	P-gp ER ^e	hERG inhibition at 10 μM (%)	brain Aβ40 reduction in mice at 10/3/1 mg/kg ^f (%)
1b	9.7	0	0.0	2.3	2.3	0.051	0.014	4	14.8	88	13/-/-
66	8.1	-1.6	1.1	1.9	0.8	0.028	0.001	28	4.9	85	89/43/-
67	7.4	-2.3	1.3	1.6	0.3	0.435	0.027	16	2.1	91	2/-/-
69	7.3	-2.4	1.7	1.9	0.2	0.054	0.009	6	1.7	85	68/34/-
109	7.3	-2.4	2.8	3.1	0.3	0.776	0.230	3			
89	7.0	-2.7	2.8	2.9	0.1	0.012	0.002	6	1.9	75	95/88/78
68	6.7	-3.0	1.6	1.7	0.1	0.077	0.025	3	2.6	65	55/26/-
88	6.3	-3.4	3.0	3.0	0	0.019	0.013	1	1.6	39	88/64/11
111	5.9	-3.8	3.1	3.1	0	0.013	0.010	1	1.5	48	93/71/41
110	5.8	-3.9	3.1	3.1	0	0.040	0.150	-4	1.5		
14d	5.8	-3.9	2.5	2.5	0	0.023	0.021	1	1.5	43	78/39/4
70	5.1	-4.6	2.3	2.3	0	0.049	0.400	-8	1.6	14	

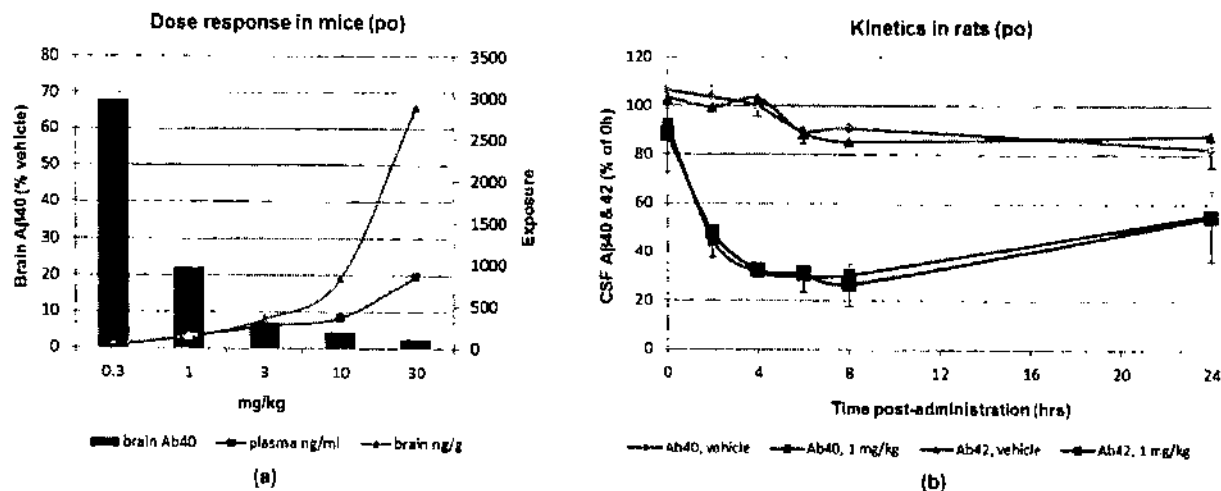


Figure 5. (a) Dose dependent inhibition of brain Aβ40 production in mice by compound 89 determined 4 h oral post dose, $n = 3-4$, mean reduction as compared to vehicle control. (b) Time dependent inhibition of CSF Aβ40/42 production in rats after an oral dose of 1 mg/kg of compound 89, $n = 2-3$. CSF was sampled at -1 and 0 h to define an average Aβ40/42 level for each individual rat; all post-treatment Aβ-levels were expressed as percentage of this pretreatment average.

outside the binding pocket (unbound geometry). As shown in Figure 4, the coordinates of bound as well as unbound 66 and 89 remain almost unchanged, therefore, the oxazine rings are without conformational strain. The geometry optimization of 109 resulted in a different ring pucker, indicating that the bound ligand is not in its lowest energy conformation. This conformational strain results in a reduced binding affinity. The geometry optimization of 110 also yields a strained conformation in the bound state. However, the effect on binding affinity is much lower than expected. A reason for this result might be the involvement of the F-substituent in the network of van der Waals interactions involving Y132, which could overcompensate the conformational energy penalty. For 67, the position of the methylene carbon next to the oxazine oxygen atom cannot be clearly assigned because of weak electron density. This is probably due to the presence of multiple ring conformations, indicating that 67 is not well bound in the active site.

The shift of cellular vs enzymatic activity depends on the pK_a : basic compounds with a $pK_a \geq 7$ show a trend for higher activity in the cell-based assay, whereas oxazines with a $pK_a < 7$ are more balanced or can even revert to have a higher potency in the enzymatic assay.⁵³ BACE1 inhibitors with enhanced basicity have been associated with increased cellular potency by favoring the acidic intracellular compartment where BACE1 is located.⁵⁴

P-gp mediated efflux is clearly correlated with the pK_a .⁵⁵ Lowering of the pK_a from 9.7 to 8.1 to 7.4 in compounds 1b, 66, and 67 resulted in a continuous reduction of the efflux ratio from 15 to 5 to 2, respectively, which remained low at a $pK_a < 7.4$. There was in general no species differences between human and mouse P-gp efflux, e.g., for compound 89 (efflux ratio = 1.9/1.9, respectively) and compound 14d (efflux ratio = 1.5/2.3, respectively).

HERG inhibition is strongly affected by the pK_a .⁵⁶ Reduction of the pK_a from 9.7 to 5.1 correlated well with hERG inhibition, which declined from 88% to 14% at 10 μ M.

The low in vivo activity of compound 1b (13% reduction of Aβ40 at 10 mg/kg) is now better understood because of the high P-gp efflux ratio of 15. Attachment of one fluorine in the

relaxed down configuration in compound 66 boosted the cellular activity 14-fold and reduced P-gp efflux 3-fold as compared to 1b, resulting in good in vivo activity (43% reduction of Aβ40 at a lower dose of 3 mg/kg). The cellular activity of compounds 88 and 111 ($IC_{50} = 0.013$ and 0.010μ M) was lower compared to 66 ($IC_{50} = 0.001 \mu$ M) but the 3-fold lower P-gp efflux of 1.6/1.5 may account for the higher in vivo activity (64 and 71% reduction of Aβ40 at 3 mg/kg) compared to 66. Most gratifyingly, the CF₃ substituted oxazine 89 with combined favorable properties (cellular $IC_{50} = 0.002 \mu$ M, P-gp efflux of 1.9) was most potent showing a reduction of Aβ40 of 78% at a dose as low as 1 mg/kg.

In Vivo Pharmacokinetics (PK) and Pharmacodynamics (PD) of Oxazine 89. The oxazine 89 was selected for further investigation of pharmacokinetic and dynamic parameters. Aβ40 lowering was determined after oral administration in wild-type mice (C57Bl/6J mice, $n = 3-4$) 4 h post dosing. Wild-type mice were used as they have a normal physiological level of APP expression and are thus expected to provide a better physiological model for Aβ-peptide production than APP-transgenic organisms. Compound 89 showed a dose-dependent inhibition of Aβ40 production in the brain of mice (Figure 5a) with a brain/plasma ratio of 1–1.5 between 0.3 and 3 mg/kg, gradually increasing to 2.3 and 3.4 at the higher doses of 10 and 30 mg/kg. In a rat PK experiment (Table 4), oxazine 89 showed a low to moderate systemic plasma clearance (16.8 mL/min/kg), a high volume of distribution (11.5 L/kg), and a long terminal half-life time (9–11 h). This, together with a good oral bioavailability (68%) and a moderately high fraction

Table 4. Pharmacokinetic Properties of Oxazine 89 in Rats^a

dose (mg/kg)	AUC _{inf} (ng·h/mL)	Cl (mL/min/kg)	Vd _{ss} (L/kg)	t _{1/2} (h)	F (%)	Fu ^c (%)
1.2 mg iv ^b	1195	16.8	11.5	8.9		
5.2 mg po ^b	3520			10.9	68	20.5

^aAll values given are means of two experiments. ^bpo: microsuspension of 89 in gelatin/aqueous NaCl; iv: solution of 89 in aqueous NaCl/N-methyl-2-pyridone. ^cFu = fraction unbound.

unbound (20.5% in plasma), would predict a favorable PD effect in rats.

Wild-type Wistar rats were used with a cannula inserted into the cisterna magna, and the kinetics of changes in the CSF A β 40/42 in an acute treatment study was followed.⁵⁷ An oral dose as low as 1 mg/kg (Figure 5b) was sufficient to induce a long lasting effect, with CSF levels remaining most inhibited 4 h post dosing and still showing a significant reduction of A β 40/42 after 24 h as a result of favorable PK properties.

The changes in plasma A β 40/42 (Figure 5b) were analyzed in relation to the measured plasma exposure in rats (Table 4). For the modeling of the plasma exposure data, a one-compartment model with first-order drug absorption and elimination was considered. For the modeling of A β 40/42 changes in plasma, an indirect response model was considered with the zero-order production rate being modulated by the pharmacological effects of the oxazine 89. The proposed model fitted the data well, and the in vivo potency was estimated to be 0.020 μ M free, which compares favorably to the in vitro measurement of IC₅₀ = 0.012 μ M in the hBACE1 enzyme assay. Thus, it can be concluded that the free plasma concentration of 89 equals the free brain concentration, which can be considered as the pharmacological relevant concentration.

In terms of its in vitro toxicological profile, compound 89 did not inhibit CYP₄₅₀ 3A4, 2D6, and 2C9 (IC₅₀ > 25 μ M) nor was there a time-dependent inhibition of CYP₄₅₀ 3A4. Furthermore, 89 was very selective against other aspartyl proteases such as human cathepsin D/E and the peptidases renin and pepsin (IC₅₀ > 200 μ M).

SUMMARY AND CONCLUSION

Starting from a very basic, in vivo marginally active 1,3-oxazine 1a, the introduction of two fluorine atoms into the headgroup reduced the pK_a considerably and led unexpectedly to good in vivo activity, as found in compound 14a. Encouraged by this finding, we performed an extensive fluorine scan to study the impact of fluorine(s) on the pK_a and a number of other parameters. Lowering the pK_a had a profound effect on enzymatic and cellular activity, hERG activity, P-gp efflux, and eventually on in vivo activity. The knowledge acquired by the fluorine headgroup scan delivered crucial information on how to improve in vivo efficacy and reduce in vitro toxicological liabilities. The CF₃-substituted oxazine 89 was found to be an orally active BACE1 inhibitor with an excellent PD profile in mice and a long lasting A β 40/42 reduction in the CSF of rats at a dose as low as 1 mg/kg. A compound from this chemical class is currently undergoing clinical evaluation for the treatment of Alzheimer's Disease.

EXPERIMENTAL SECTION

General. All solvents and reagents were obtained from commercial sources and were used as received. All reactions were followed by TLC (TLC plates F254, Merck) or LCMS (liquid chromatography–mass spectrometry) analysis. Silica gel chromatography was either performed using cartridges packed with silica gel (ISOLUTE Columns, TELOS Flash Columns) or silica-NH₂ gel (TELOS Flash NH₂ Columns) on ISCO Combi Flash Companion or on glass columns on silica gel 60 (32–60 mesh, 60 Å). Proton NMR spectra were obtained on Bruker Avance 300, 400, or 600 MHz spectrometer with chemical shifts (δ in ppm) reported relative to tetramethylsilane or the residual solvent peak as the internal reference (i.e., CDCl₃ = 7.26 ppm, DMSO-*d*₆ = 2.50 ppm) as the internal reference. ¹H resonances are reported to the nearest 0.01 ppm. NMR abbreviations are as follows: s,

singlet; d, doublet; t, triplet; q, quadruplet; quint, quintuplet; sext, sextuplet; m, multiplet; br, broadened. Coupling constants (*J*) are reported to the nearest 0.1 Hz. Purity was analyzed by reverse phase HPLC and for specific compounds by elemental analysis. HPLC was performed on Finnigan LTQ (Thermo Fisher Scientific) and Agilent RRLC 1200 equipment. Column: Agilent XDB C15, 30 mm $\times$ 4.6 mm, 3.5 μ m. Analytical conditions: gradient used, 5–95% acetonitrile in water containing 0.1% trifluoroacetic acid in 3 min. Flow: 4.5 mL/min. UV-detector: DAD 190–400 nm. Sample solvent: in water/acetonitrile (8/2). The UV detection was an averaged signal from wavelengths of 190–400 nm. Elemental analyses were performed by Solvias AG (Mattenstrasse, Postfach, CH-4002 Basel, Switzerland). Mass spectra were recorded on an SSQ 7000 (Finnigan-MAT) spectrometer for electron impact ionization. The purities of final test compounds as measured by HPLC were found to be above 95%. Melting points were determined on a Büchi melting point B-540: heating rate 1 °C/min starting 15 °C below melting point. LC-HRMS spectra were recorded with an Agilent LC-system consisting of an Agilent 1290 high pressure system, a CTC PAL auto sampler, and an Agilent 6520 QTOF. The separation was achieved on a Zorbax Eclipse Plus C18 1.7 μ m 2.1 mm $\times$ 30 mm column at 55 °C; eluent A = 0.01% formic acid in water; B = 0.01% formic acid in acetonitrile:2-propanol (8:2); flow, 1 mL/min. Gradient: 0 min 5% of B, 0.3 min 5% of B, 4.5 min 99% of B, 5 min 99% of B. The injection volume was 2 μ L. All assay compounds had a purity of >95%. LC-MS (ESI, positive or negative ion) data were recorded on Waters UPLC-MS Systems equipped with Waters Acquity, a CTC PAL auto sampler, and a Waters SQD single quadrupole mass spectrometer using ES ionization modes (positive and/or negative). The separation was achieved on a Zorbax Eclipse Plus C18 1.7 μ m 2.1 mm $\times$ 30 mm column at 50 °C; A = 0.01% formic acid in water, B = acetonitrile at flow 1; gradient: 0 min 3%B, 0.2 min 3%B, 2 min 97%B, 1.7 min 97%B, 2.0 min 97%B. The injection volume was 2 μ L. MS (ESI, positive or negative ion): FIA (flow injection analysis)-MS were recorded on a Applied Biosystem API150 mass spectrometer. Sample introduction was made with a CTC PAL auto sampler and a Shimadzu LC-10ADVP pump. The samples were directly flushed to the ESI source of the mass spectrometer with a flow 50 μ L/min of a mixture of acetonitrile and 10 mM ammonium acetate (1:1) without a column. The injection volume was 2 μ L.

(R)-2-Methyl-propane-2-sulfinic Acid [1-(2-Fluoro-5-nitro-phenyl)-(E)-ethylidene]-amide (3).³² To a solution of the (R)-(+)-tert-butylsulfonamide (10.88 g, 89.8 mmol) in THF (400 mL) was added subsequently 1-(2-fluoro-5-nitro-phenyl)-ethanone (2) (16.45 g, 89.8 mmol) and titanium(IV)ethoxide (40.70 g, 178.4 mmol), and the solution was stirred at reflux temperature for 5 h. The mixture was cooled to 22 °C and treated with brine (400 mL), and the suspension was stirred for 10 min and filtered over Dicalite. The layers were separated, the aqueous layer was extracted with EtOAc, and the combined organic layers were washed with water, dried, and evaporated. The residue was purified by flash column chromatography on silica gel using a mixture of cyclohexane/EtOAc (2:1) to give the title compound 3 (21.56 g, 84%) as a pale-yellow solid: mp 83–85 °C. ¹H NMR (600 MHz, CDCl₃) δ ppm 8.57 (dd, *J* = 6.3, 2.9 Hz, 1 H), 8.33 (ddd, *J* = 9.0, 3.2, 3.1 Hz, 1 H), 7.30 (t, *J* = 9.5 Hz, 1 H), 2.82 (d, *J* = 3.5 Hz, 3 H), 1.34 (s, 9 H). MS (ISP): *m/z* = 287.0 [*M* + *H*]⁺. Anal. (C₁₂H₁₃FN₂O₃S) Calcd: C 50.34%, H 5.28%, N 9.78%, S 11.20%. Found: C 50.49%, H 5.02%, N 9.94%, S 11.31%.

(R)-2,2-Difluoro-3-(2-fluoro-5-nitro-phenyl)-3-((R)-2-methyl-propane-2-sulfinylamino)-butyric Acid Ethyl Ester (5).³² In a dry apparatus, a suspension of freshly activated zinc powder (37.5 g, 574 mmol) and CuCl (5.99 g, 60.5 mmol) in dry THF (100 mL) was heated under an inert atmosphere to reflux for 30 min. The suspension was cooled to 22 °C and treated with a solution of ethyl 2-bromo-2,2-difluoroacetate (30.7 g, 151 mmol) in dry THF (50 mL) over 1 h, and stirring was continued for 30 min. The black mixture was treated with a solution of (R)-2-methyl-propane-2-sulfinic acid [1-(2-fluoro-5-nitro-phenyl)-(E)-ethylidene]-amide (3) (17.5 g, 60.5 mmol) in THF (50 mL), and stirring was continued at 22 °C for 2 h. The suspension was filtered through Dicalite and washed with *t*-butyl methyl ether. The

combined filtrates were subsequently washed with aqueous citric acid (5%) and aqueous saturated NaHCO_3 solution, the organic layer was dried over Na_2SO_4 and filtered, and the filtrate was evaporated. The residue was purified by flash column chromatography on silica gel using a mixture of *n*-heptane/EtOAc (2:1) to give the title compound **5** (17.24 g, 69%) as a yellow oil. ^1H NMR (300 MHz, CDCl_3) δ ppm 8.51 (dd, J = 6.6, 2.7 Hz, 1 H), 8.29 (ddd, J = 9.1, 3.8, 3.0 Hz, 1 H), 7.26 (dd, J = 11.3, 9.1 Hz, 1 H), 4.72 (s, 1 H), 4.34 (qd, J = 7.2, 1.5 Hz, 2 H), 2.09–2.17 (m, 3 H), 1.32 (t, J = 7.3 Hz, 3 H), 1.32 (s, 9 H). MS (ISP): m/z = 411.2 $[\text{M} + \text{H}]^+$. Calcd: $\text{C}_{16}\text{H}_{11}\text{F}_3\text{N}_3\text{O}_5\text{S}$ Calcd: C 46.82%, H 5.16%, N 6.83%, S 7.81%. Found: C 46.73%, H 4.89%, N 6.81%, S 7.66%. The configuration of the newly introduced stereogenic center was confirmed by X-ray crystallographic analysis of the target compound **14d** complexed with BACE1 (3ZMG).

(R)-2-Methyl-propane-2-sulfinic Acid [(R)-2,2-Difluoro-1-(2-fluoro-5-nitro-phenyl)-3-hydroxy-1-methyl-propyl]-amide (7).³² To a solution of (R)-2,2-difluoro-3-(2-fluoro-5-nitro-phenyl)-3-((R)-2-methyl-propane-2-sulfinylamino)-butyric acid ethyl ester (**5**) (3.78 g, 9.2 mmol) in dry THF (24 mL) was added at 0 °C lithium borohydride (9.0 mmol), and stirring was continued at 0 °C for 1.5 h. The reaction mixture was quenched by addition of acetic acid (1 mL) and water, the mixture was extracted with EtOAc, and the organic layers were washed with brine, dried over Na_2SO_4 , and evaporated. The residue was purified by flash column chromatography on silica gel using a mixture of *n*-heptane/EtOAc (3:2) to give the title compound **7** (2.90 g, 86%) as a pale-yellow solid. ^1H NMR (600 MHz, CDCl_3) δ ppm 8.46 (dd, J = 6.8, 2.8 Hz, 1 H), 8.25 (ddd, J = 9.0, 3.7, 2.9 Hz, 1 H), 7.25 (dd, J = 11.7, 9.0 Hz, 1 H), 5.36 (br s, 1 H), 4.51–5.03 (br s, 1 H), 4.21 (ddd, J = 25.0, 13.4, 2.7 Hz, 1 H), 3.84 (td, J = 12.8, 7.9 Hz, 1 H), 2.19 (s, 3 H), 1.31 (s, 9 H). MS (ISP): m/z = 369.0 $[\text{M} + \text{H}]^+$. A small sample was recrystallized from EtOAc/*n*-heptane: mp 154–155 °C. Anal. ($\text{C}_{14}\text{H}_{19}\text{F}_3\text{N}_3\text{O}_4\text{S}$) Calcd: C 45.65%, H 5.20%, N 7.60%, S 8.70%. Found: C 45.60%, H 5.07%, N 7.79%, S 8.78%.

(R)-3-Amino-2,2-difluoro-3-(2-fluoro-5-nitro-phenyl)-butan-1-ol (9).³² A solution of (R)-2-methyl-propane-2-sulfinic acid [(R)-2,2-difluoro-1-(2-fluoro-5-nitro-phenyl)-3-hydroxy-1-methyl-propyl]-amide (**7**) (3.79 g, 10.3 mmol) in MeOH (50 mL) was treated with a solution of HCl in 1,4-dioxane (4 M, 13 mL), and stirring was continued at 22 °C for 1 h. The mixture was evaporated, the residue was partitioned between EtOAc and aqueous Na_2CO_3 solution (2 M), and the organic layers were washed with brine, dried over Na_2SO_4 , filtered, and evaporated to give the crude title compound **9** (2.50 g, 92%) as a pale-yellow solid, which was used without further purification. ^1H NMR (600 MHz, CDCl_3) δ ppm 8.49 (dd, J = 7.0, 2.8 Hz, 1 H), 8.25 (ddd, J = 9.0, 3.8, 2.9 Hz, 1 H), 7.25 (dd, J = 11.5, 9.0 Hz, 1 H), 4.02 (ddd, J = 19.7, 12.7, 8.6 Hz, 1 H), 3.86 (dd, J = 23.5, 11.8 Hz, 1 H), 2.01–3.63 (3 H), 1.85 (d, J = 0.9 Hz, 3 H). MS (ISP): m/z = 265.1 $[\text{M} + \text{H}]^+$. A small sample was triturated with 2-propanol: mp 110–111 °C. Anal. ($\text{C}_{10}\text{H}_{11}\text{F}_3\text{N}_3\text{O}_3$) Calcd: C 45.46%, H 4.20%, N 10.60%. Found: C 45.67%, H 4.20%, N 11.03%.

(R)-5,5-Difluoro-4-(2-fluoro-5-nitro-phenyl)-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine (11).³³ To a solution of (R)-3-amino-2,2-difluoro-3-(2-fluoro-5-nitro-phenyl)-butan-1-ol (**9**) (1.5 g, 5.7 mmol) in EtOH (30 mL) at 22 °C was added under argon cyanogen bromide (0.91 g, 8.6 mmol), and the light-yellow solution was stirred in a sealed tube at 85 °C for 24 h. The mixture was cooled to 22 °C, evaporated, and the residue partitioned between EtOAc and saturated aqueous Na_2CO_3 solution. The organic layers were dried over Na_2SO_4 , filtered, and evaporated, and the residue was purified by flash column chromatography on silica gel using a mixture of *n*-heptane/EtOAc (5:2) to afford the title compound **11** (1.30 g, 79%) as a pale-yellow solid. ^1H NMR (600 MHz, CDCl_3) δ ppm 8.59 (dd, J = 6.6, 2.9 Hz, 1 H), 8.21 (ddd, J = 9.0, 3.8, 3.0 Hz, 1 H), 7.20 (dd, J = 10.6, 8.9 Hz, 1 H), 4.25–4.45 (2 H), 4.27 (ddd, J = 23.2, 11.8, 3.8 Hz, 1 H), 4.06 (ddd, J = 11.7, 10.4, 8.2 Hz, 1 H), 1.79 (t, J = 2.3 Hz, 3 H). MS (ISP): m/z = 290.2 $[\text{M} + \text{H}]^+$. A small sample was recrystallized from EtOAc/*n*-heptane: mp 134–135 °C. Anal. ($\text{C}_{11}\text{H}_{10}\text{F}_3\text{N}_3\text{O}_3$) Calcd: C 45.68%, H 3.49%, N 14.53%. Found: C 45.63%, H 3.41%, N 14.65%.

(R)-4-(5-Amino-2-fluoro-phenyl)-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine (13).⁴³ To a solution of (R)-5,5-difluoro-4-(2-fluoro-5-nitro-phenyl)-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine **11** (1.29 g, 4.47 mmol) in EtOH (35 mL) was added at 22 °C under an inert atmosphere palladium on carbon (10% Pd, 238 mg), and the mixture was stirred under a hydrogen atmosphere (balloon) at 22 °C for 1 h. The catalyst was filtered off and washed twice with EtOH, and the solvent was removed under reduced pressure to give the crude title compound **13** (1.14 g, 98%) as a colorless foam which was used without further purification. ^1H NMR (600 MHz, CDCl_3) δ ppm 6.85 (dd, J = 11.7, 8.6 Hz, 1 H), 6.79 (dd, J = 6.5, 3.0 Hz, 1 H), 6.58 (ddd, J = 8.6, 3.2, 3.1 Hz, 1 H), 4.10–4.90 (br s, 2 H), 4.10 (ddd, J = 13.2, 11.5, 7.5 Hz, 1 H), 3.99 (ddd, J = 18.2, 11.6, 5.9 Hz, 1 H), 3.55 (br s, 2 H), 1.75 (t, J = 2.3 Hz, 3 H). MS (ISP): m/z = 260.1 $[\text{M} + \text{H}]^+$. A small sample was purified by flash column chromatography on silica gel using a mixture of dichloromethane/methanol (19:1) containing 1% of ammonium hydroxide, followed by crystallization from *tert*-butylmethyl ether: mp 122–123 °C. Anal. ($\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_3\text{O}$) Calcd: C 50.97%, H 4.67%, N 16.21%. Found: C 51.03%, H 4.52%, N 16.34%.

General Procedure for the Synthesis of the Amides 14a and 14d. To a solution of the carboxylic acid (0.23 mmol) in MeOH (5 mL) at 0 °C was added 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholinium chloride hydrate (DMTMM) (80 mg, 0.27 mmol). The colorless solution was stirred at 0 °C for 30 min, then a solution of (R)-4-(5-amino-2-fluoro-phenyl)-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine (**13**) (54 mg, 0.21 mmol) in MeOH (5 mL) was added dropwise at 0 °C and the mixture was stirred at 22 °C for 20 h. The mixture was partitioned between aqueous Na_2CO_3 solution (1 M) and dichloromethane, and the organic layers were washed with brine, dried over Na_2SO_4 , and evaporated. The residue was purified by flash column chromatography on silica gel using a mixture of methanol/methylene chloride (0–10%) to give the pure amides **14a** and **14d**.

5-Chloro-pyridine-2-carboxylic Acid [3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl]-amide (14a).⁴³ The condensation of **13** (110 mg, 0.42 mmol) and 5-chloro-pyridine-2-carboxylic acid (67 mg, 0.42 mmol) yielded the title compound **14a** (122 mg, 72%) as a colorless solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 10.70 (s, 1 H), 8.79 (d, J = 2.0 Hz, 1 H), 8.13–8.22 (m, 2 H), 7.95 (dd, J = 7.1, 2.6 Hz, 1 H), 7.83–7.91 (m, 1 H), 7.15 (dd, J = 11.8, 8.8 Hz, 1 H), 4.29 (ddd, J = 17.8, 12.0, 5.8 Hz, 1 H), 4.02 (ddd, J = 17.8, 11.8, 5.8 Hz, 1 H), 3.30 (s, 2 H), 1.64 (s, 3 H). LC-HRMS: m/z 399.0839 $[(\text{M} + \text{H})^+]$ calcd for $\text{C}_{17}\text{H}_{15}\text{ClF}_3\text{N}_4\text{O}_2$, 399.0830. MS (ISP): m/z = 399.2 $[\text{M} + \text{H}]^+$.

5-Cyano-pyridine-2-carboxylic Acid [3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl]-amide (14d).⁴³ The condensation of **13** (80 mg, 0.31 mmol) and 5-cyano-pyridine-2-carboxylic acid (50 mg, 0.34 mmol) yielded the title compound **14d** (78 mg, 65%) as a colorless solid. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ ppm 10.86 (s, 1 H), 9.20 (dd, J = 2.1, 0.9 Hz, 1 H), 8.58 (dd, J = 8.2, 2.1 Hz, 1 H), 8.28 (dd, J = 8.2, 0.8 Hz, 1 H), 7.98 (dd, J = 7.2, 2.8 Hz, 1 H), 7.88 (ddd, J = 8.8, 3.8, 2.9 Hz, 1 H), 7.17 (dd, J = 11.8, 8.8 Hz, 1 H), 5.91 (br s, 2 H), 4.30 (ddd, J = 18.5, 11.9, 5.4 Hz, 1 H), 4.03 (ddd, J = 17.4, 11.8, 5.7 Hz, 1 H), 1.65 (s, 3 H). LC-HRMS: m/z 390.1183 $[(\text{M} + \text{H})^+]$ calcd for $\text{C}_{18}\text{H}_{15}\text{F}_3\text{N}_5\text{O}_2$, 390.1172. MS (ISP): m/z = 390.2 $[\text{M} + \text{H}]^+$. X-ray PDB accession code: 3ZMG.

3-Methyl-5-(trifluoromethyl)-4,5-dihydroisoxazole (76). To diethyl ether (640 mL) was added subsequently at –78 °C 3,3,3-trifluoroprop-1-ene (65.3 g, 680 mmol), nitroethane (30.0 g, 400 mmol), Et_3N (405 mg, 4 mmol), and phenyl isocyanate (97.2 g, 816 mmol), the solution was warmed to 22 °C, and stirring was continued for 74 h. The suspension was filtered and the filtrate distilled from bulb to bulb at 50–60 °C/1.0 mbar to give a 9:1 mixture of the title compound **76** and its corresponding position isomer (37.3 g, 61%) as a pale-yellow liquid. ^1H NMR (600 MHz, CDCl_3) δ ppm 4.80–4.87 (m, 1 H), 3.24 (ddq, J = 17.8, 11.5, 1.0, 1.0, 1.0 Hz, 1 H), 3.08 (ddq, J = 17.8, 5.8, 0.9, 0.9, 0.9 Hz, 1 H), 2.04 (t, J = 1.1 Hz, 3 H). ^1H NMR signals of position isomer 3-methyl-4-(trifluoromethyl)-4,5-dihydrois-

soxazole: 4.44–4.53 (m, 2 H), 3.83–3.91 (m, 1 H), 2.12 (t, $J = 0.9$ Hz, 3 H). MS: $m/z = 153$ [M] $^+$. Anal. ($C_7H_6F_3NO$) Calcd: C 39.22%, H 3.95%, N 9.15%. Found: C 39.27%, H 3.60%, N 9.34%.

(3S,5S)-3-(2-Fluoro-phenyl)-3-methyl-5-trifluoromethyl-isoxazolidine (79). To a stirred solution of 1-bromo-2-fluoro-benzene (77) (11.2 g, 64 mmol) in THF (50 mL) and toluene (140 mL) was added at -78 °C n -BuLi (1.6 M in hexane, 38 mL, 61 mmol), keeping the temperature below -70 °C, and stirring was continued at -78 °C for 1 h. To a solution of 3-methyl-5-(trifluoromethyl)-4,5-dihydroisoxazole (76) (4.68 g, 30.6 mmol) in toluene (330 mL) was added at -78 °C $BF_3 \cdot Et_2O$ (8.68 g, 61.1 mmol), which was followed by the addition of the phenyl lithium reagent prepared above using an insulated cannula over 10 min keeping the temperature below -70 °C. The mixture was stirred at -78 °C for 1 h, quenched with saturated aqueous NH_4Cl , and extracted with EtOAc. The organic layers were washed with brine, dried, and evaporated, and the residue was purified by flash column chromatography on silica gel using a mixture of n -heptane/EtOAc (50:1) to give the racemic title compound 79 (4.275 g, 56%) as a pale-yellow liquid. The racemate was resolved on a chiral HPLC column (Chiralpak AD) using n -heptane/EtOH (95:5) to give the desired (3S,5S)-3-(2-fluoro-phenyl)-3-methyl-5-trifluoromethyl-isoxazolidine (79) (1.719 g, 23%) as the slower eluting enantiomer with positive optical rotation and an $ee > 99.5\%$. 1H NMR (300 MHz, $CDCl_3$) δ ppm 7.78 (td, $J = 8.0, 1.3$ Hz, 1 H), 7.22–7.31 (m, 1 H), 7.14 (td, $J = 7.7, 1.2$ Hz, 1 H), 7.05 (ddd, $J = 12.0, 8.1, 1.1$ Hz, 1 H), 5.67 (s, 1 H), 4.27 (sxt, $J = 7.4$ Hz, 1 H), 3.10 (ddd, $J = 13.4, 8.2, 1.0$ Hz, 1 H), 2.37 (dd, $J = 13.3, 7.5$ Hz, 1 H), 1.62 (s, 3 H). MS: $m/z = 250.4$ [$M + H$] $^+$. The relative and absolute configuration of the newly introduced stereogenic centers were confirmed by X-ray crystallographic analysis of the target compound 89 complexed with BACE1 (4J1F). (3R,5R)-3-(2-Fluoro-phenyl)-3-methyl-5-trifluoromethyl-isoxazolidine (1.738 g, 23%) was obtained as the faster eluting enantiomer with negative optical rotation and an $ee > 99.5\%$.

(2S,4S)-4-Amino-1,1,1-trifluoro-4-(2-fluorophenyl)pentan-2-ol (81). To a solution of (3S,5S)-3-(2-fluoro-phenyl)-3-methyl-5-trifluoromethyl-isoxazolidine (79) (1.50 g, 6.02 mmol) in EtOH (35 mL) was added 22 °C ammonium formate (3.04 g, 48.2 mmol) and Pd/C (10%, 320 mg), and stirring of the mixture was continued at 22 °C for 4 h. The suspension was filtered, the filtrate was evaporated, and the residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The organic layer was dried and evaporated to give the crude title compound (1.50 g, 99%) as a colorless solid which was used without further purification. 1H NMR (300 MHz, $CDCl_3$) δ ppm 7.45 (td, $J = 8.1, 1.8$ Hz, 1 H), 7.28–7.37 (m, 1 H), 7.19 (td, $J = 7.5, 1.2$ Hz, 1 H), 7.09 (ddd, $J = 12.6, 8.1, 1.3$ Hz, 1 H), 3.61 (dq, $J = 11.4, 6.7, 6.7, 2.0$ Hz, 1 H), 2.50 (dt, $J = 14.3, 1.6$ Hz, 1 H), 1.75–2.75 (br, 2 H), 1.82 (dd, $J = 14.3, 11.5$ Hz, 1 H), 1.69 (d, $J = 1.2$ Hz, 3 H) (OH not detectable). MS: $m/z = 252.2$ [$M + H$] $^+$. A small sample was purified by flash column chromatography on silica gel using a mixture of n -heptane/EtOAc: mp 113–115 °C. Anal. ($C_{11}H_{13}F_4NO$) Calcd: C 52.59%, H 5.22%, N 5.58%. Found: C 52.49%, H 5.24%, N 5.73%.

(4S,6S)-4-(2-Fluorophenyl)-4-methyl-6-(trifluoromethyl)-5,6-dihydro-4H-1,3-oxazin-2-amine (83). To a solution of (2S,4S)-4-amino-1,1,1-trifluoro-4-(2-fluorophenyl)pentan-2-ol (81) (1.834 g, 7.30 mmol) in EtOH (38 mL) was added a solution of Br-CN (5 M in CH_3CN , 2.2 mL, 11.0 mmol), and the mixture was stirred in a sealed tube at 85 °C for 15 h. The mixture was evaporated and the residue partitioned between EtOAc and saturated aqueous Na_2CO_3 solution, and the organic layer was dried and evaporated, and the residue was purified by chromatography (silica- NH_2) using a mixture of n -heptane/EtOAc (5:1 to 0:1) to afford the title compound 83 (817 mg, 41%) as a colorless oil. 1H NMR (600 MHz, $CDCl_3$) δ ppm 7.38 (td, $J = 8.1, 1.9$ Hz, 1 H), 7.24–7.29 (m, 1 H), 7.13 (td, $J = 7.6, 1.3$ Hz, 1 H), 7.04 (ddd, $J = 12.4, 8.1, 1.3$ Hz, 1 H), 4.35 (br s, 2 H), 3.97 (dq, $J = 12.4, 6.0, 6.0, 2.8$ Hz, 1 H), 2.77 (dd, $J = 13.6, 2.8$ Hz, 1 H), 1.86 (dd, $J = 13.6, 12.6$ Hz, 1 H), 1.63 (d, $J = 1.3$ Hz, 3 H). MS: $m/z = 277.1$ [$M + H$] $^+$. A small sample was subjected again to flash column chromatography on silica- NH_2 gel using a mixture of n -

heptane/EtOAc: Anal. ($C_{12}H_{14}F_4N_2O$) Calcd: C 52.18%, H 4.38%, N 10.14%. Found: C 52.04%, H 4.14%, N 10.05%.

(4S,6S)-4-(2-Fluoro-5-nitrophenyl)-4-methyl-6-(trifluoromethyl)-5,6-dihydro-4H-1,3-oxazin-2-amine (85). To concentrated sulfuric acid (11 mL) was added portion wise (4S,6S)-4-(2-fluorophenyl)-4-methyl-6-(trifluoromethyl)-5,6-dihydro-4H-1,3-oxazin-2-amine (83) (760 mg, 2.75 mmol) at 22 °C, the solution obtained was cooled to 0 °C and treated with red fuming HNO_3 (0.17 mL, 3.85 mmol) over 20 min, and stirring was continued at 0 °C for 30 min. The reaction mixture was slowly added to crushed ice/water (150 mL), the pH was adjusted to 10 using NaOH, the aqueous layer was extracted with EtOAc, the organic layer was dried and evaporated, and the residue was chromatographed on silica gel using a mixture of n -heptane/EtOAc (3:1) to afford the title compound 85 (800 mg, 91%) as a pale-yellow oil. 1H NMR (300 MHz, $CDCl_3$) δ ppm 8.37 (dd, $J = 7.0, 2.9$ Hz, 1 H), 8.19 (ddd, $J = 8.9, 4.0, 3.0$ Hz, 1 H), 7.21 (dd, $J = 10.7, 8.9$ Hz, 1 H), 4.31 (br s, 2 H), 3.87–4.01 (m, 1 H), 2.78 (dd, $J = 13.8, 2.7$ Hz, 1 H), 1.95 (dd, $J = 13.8, 12.8$ Hz, 1 H), 1.64 (d, $J = 1.2$ Hz, 3 H). MS: $m/z = 322.5$ [$M + H$] $^+$.

(4S,6S)-4-(5-Amino-2-fluorophenyl)-4-methyl-6-(trifluoromethyl)-5,6-dihydro-4H-1,3-oxazin-2-amine (87). A suspension of (4S,6S)-4-(2-fluoro-5-nitrophenyl)-4-methyl-6-(trifluoromethyl)-5,6-dihydro-4H-1,3-oxazin-2-amine (85) (765 mg, 2.38 mmol) in EtOH (45 mL) and Et_3N (0.33 mL, 2.38 mmol) was treated with Pd/C (10%, 127 mg), and the mixture was hydrogenated at atmospheric pressure and 22 °C for 3 h. The mixture was filtered and the filtrate evaporated to give the crude title compound 87 (680 mg, 98%) as a colorless foam which was used without further purification. 1H NMR (300 MHz, $CDCl_3$) δ ppm 6.83 (dd, $J = 11.7, 8.5$ Hz, 1 H), 6.67 (dd, $J = 6.9, 2.8$ Hz, 1 H), 6.52 (ddd, $J = 8.5, 3.0, 3.0$ Hz, 1 H), 4.04 (dq, $J = 12.3, 6.0, 6.0, 2.8$ Hz, 1 H), 3.90–4.60 (br s, 2 H), 3.57 (br s, 2 H), 2.76 (dd, $J = 13.5, 2.8$ Hz, 1 H), 1.81 (dd, $J = 13.4, 12.6$ Hz, 1 H), 1.60 (d, $J = 1.2$ Hz, 3 H). MS: $m/z = 292.5$ [$M + H$] $^+$. Anal. ($C_{12}H_{13}F_4N_2O$) Calcd: C 49.49%, H 4.50%, N 14.43%. Found: C 49.25%, H 4.51%, N 14.15%.

N-(3-(4S,6S)-2-Amino-4-methyl-6-(trifluoromethyl)-5,6-dihydro-4H-1,3-oxazin-4-yl)-4-fluorophenyl)-5-cyanopicolinamide (89). The coupling of (4S,6S)-4-(5-amino-2-fluorophenyl)-4-methyl-6-(trifluoromethyl)-5,6-dihydro-4H-1,3-oxazin-2-amine (87) and 5-cyano-pyridine-2-carboxylic acid (65), carried out according to the general procedure for 14a and 14d, yielded a crude material which was purified by chromatography (silica- NH_2) using a mixture of n -heptane/EtOAc (1:1) to give the title compound 89 (69% yield) as a colorless solid. 1H NMR (300 MHz, $CDCl_3$) δ ppm 9.84 (s, 1 H), 8.89 (dd, $J = 2.0, 0.8$ Hz, 1 H), 8.43 (dd, $J = 8.1, 0.8$ Hz, 1 H), 8.20 (dd, $J = 8.3, 2.0$ Hz, 1 H), 7.99 (ddd, $J = 8.7, 4.1, 2.9$ Hz, 1 H), 7.48 (dd, $J = 7.1, 2.8$ Hz, 1 H), 7.11 (dd, $J = 11.4, 8.8$ Hz, 1 H), 4.24 (br s, 2 H), 4.03 (dq, $J = 12.2, 6.0, 6.0, 2.6$ Hz, 1 H), 2.81 (dd, $J = 13.7, 2.6$ Hz, 1 H), 1.89 (dd, $J = 13.6, 12.8$ Hz, 1 H), 1.64 (d, $J = 1.0$ Hz, 3 H). LC-HRMS m/z 422.1243 [($M + H$) $^+$ calcd for $C_{19}H_{16}F_4N_5O_2$, 422.1235]. MS: $m/z = 422.5$ [$M + H$] $^+$. X-ray PDB accession code: 4J1F.

BACE1 and BACE2 Enzyme Assay. Enzyme inhibition assays were performed in 384-well microtiter plates (black with clear bottom, nonbinding surface from Corning, cat. no. 3655) in a final volume of 51 μ L. Test compounds dissolved in DMSO at a concentration of 10 mM were serially diluted in DMSO (15 concentrations, 1/3 dilution steps, final concentration range: 200–0.0004 μ M). Then 1 μ L of diluted compounds were mixed with 40 μ L of enzyme on an H&P Teleshaker for 4 min. After addition of the MR121-labeled substrate, the plates were again strongly shaken for 2 min. The enzymatic reaction was followed by reading the fluorescence emission on a plate: vision reader (Perkin-Elmer) (excitation wavelength, 630 nm; emission, 695 nm) for at least 30 min in a kinetic measurement detecting an increase of MR121 fluorescence following the cutting of the quenched substrate. The slope in the linear range of the kinetic was calculated and the IC_{50} of the test compounds were determined using a four parameter Hill equation for curve fitting on the calculated % inhibition values referenced to wells without enzyme and wells with enzyme but no test compound, respectively. BACE1 enzyme used in

the assay was prepared and purified as described.⁵⁸ The preparation of BACE2 was carried out following exactly the known protocol.⁴⁶ BACE1/2 assay: The final enzyme concentration in the assay was 30 and 125 nM, respectively. The assay buffer was 100 mM sodium acetate, 20 mM EDTA, 0.05% BSA, pH 4.0. The substrate peptide was of sequence WSEVNLDAEFRG-MR121 and used at a final concentration of 300 nM.

Cathepsin D Enzyme Assay. Cathepsin D from human liver was purchased from Calbiochem (cat. no. 219401) and used at a final concentration of 200 nM. The assay buffer was 100 mM sodium acetate at pH 5.5 with 0.05% BSA. The labeled substrate peptide was of sequence WTSVLMAAPC-MR121 (measurement time >60 min).

Cathepsin E Enzyme Assay. Recombinant human cathepsin E was purchased from R&D Systems (cat. no. 1294-AS) and used at a final concentration of 0.01 nM. The assay buffer was 100 mM sodium acetate at pH 4.5 with 0.05% BSA. The labeled substrate was of sequence MR121-CKLVFFAEDW.

Renin Enzyme Assay. Renin was purchased from Anaspec (cat. no. 72041) and used at a final concentration of 5 nM. The assay buffer was 50 mM MES at pH 6.2 with 0.5% BSA and 2 mM EDTA. The substrate peptide was of sequence WIPHPFLVIHTC-MR121.

Pepsin Enzyme Assay. Pepsin from porcine stomach mucosa was purchased from Calbiochem (cat. no. 516360) and used at a final concentration of 8 nM. The assay buffer was 100 mM sodium acetate at pH 4.0 with 0.05% BSA, and the substrate was the same as for cathepsin D. All substrate peptides were prepared and purified by Biosyntan, Berlin, Germany, and purchased as lyophilized powders. After reconstitution in DMSO small aliquots were kept at -20 °C.

Cell-Based Assay. HEK293 cells transfected with wild-type human APP cDNA were seeded in 96-well Microtiter plates in cell culture medium (Iscove's, plus 10% (v/v) fetal bovine serum, penicillin/streptomycin) to about 80% confluency, and the compounds were added at a 3X concentration in 1/3 volume of culture medium (final DMSO concentration was kept at 1% v/v). After 18–20 h incubation at 37 °C and 5% CO₂ in a humidified incubator, the culture supernatants were harvested for the determination of A β 40 concentrations by AlphaLISA technique using PerkinElmer Human Amyloid β 1–40 kit (A β 1–40, high specificity, cat. no. AL275 C/F). In a PerkinElmer OptiPlate–384 (cat. no. 6007290), 6 μ L of culture supernatants were added to 2 μ L of AlphaLISA Anti hu A β acceptor beads (50 μ g/mL), 5 nM Biotinylated Antibody Anti A β 1–40, 1X AlphaLISA HiBlock buffer solution and incubated for 1 h at room temperature. Then 16 μ L of a Streptavidin Donor beads (250 ng/mL in 1X AlphaLISA solution) were added, and the plate was incubated for 30 min at room temperature. Light emission at 615 nm was then recorded on an EnVision-2104 multilabel reader. Levels of A β 40 in the culture supernatants were calculated as percentage of maximum signal (cells treated with 1% DMSO without inhibitor). The IC₅₀ values were calculated using the Excel XLfit software.

P-gp Assay. Bidirectional transport was examined in LLC-PK1 cells (Lewis-lung cancer porcine kidney 1 L, ATCC CL-101) stably expressing human MDR1. The cell lines were obtained from Dr. Alfred Schinkel, The Netherlands Cancer Institute (Amsterdam, The Netherlands) and used under a license agreement. Both cell lines were cultured on semipermeable insert plates (Costar, surface area 0.33 cm², pore size 3.0 μ m), and transport experiments were performed on day 4 after seeding using medium without phenol red. Bidirectional transport was assessed in triplicate inserts with or without inhibitor (elacridar, 1 μ M), and the cell monolayer integrity was assessed via the permeability of lucifer yellow (10 μ M). Before each assay, the culture medium in the receiver compartment was replaced with fresh medium. Bidirectional transport was then initiated by the addition of medium consisting of test compound (1 μ M) and lucifer yellow to the donor compartments. The plates were incubated for 3.5 h at 37 °C, 5% CO₂, and air-saturated humidity. At the end of incubation, samples were taken from the donor and the receiver compartments, and the levels of each compound were determined either by LC-MS/MS or scintillation counting. Lucifer yellow in the receiver samples was quantified using a fluorescence-based plate reader set at excitation and emission wavelengths of 430 and 535 nm,

respectively. Experiments showing lucifer yellow permeation superior to 1%/h were rejected. The apparent permeability coefficient (P_{app}) was calculated using the following equation: $P_{app} = 1/(A \cdot C_0) \cdot (dQ/dt)$, where dQ/dt is the amount of compound transported over time, A is the surface area of the transwell, and C_0 is the initial concentration of the test compound. Mean P_{app} value and standard deviation were calculated from triplicate inserts. Active efflux by MDR1 was assessed by determining the vectorial transport or efflux ratio (ER), calculated from the basolateral-to-apical P_{app} divided by the apical-to-basolateral P_{app} . Digoxin was included in each experiment as a positive control.

HERG Inhibition. HERG inhibition was tested as described.⁵⁹

Protein Crystallography. X-ray crystal structures of BACE1 and BACE2 were determined as described by Kuglstatter et al.⁶⁰ and Ruf et al.⁴⁸

Pharmacokinetic Experiments in Mice and Rats. Animals and Housing Conditions. Animals were maintained in a 12/12 h light/dark cycle, with lights starting at 6 a.m., and experiments were conducted during the light phase. Animal housing and experimental procedures were in line with ethical and legal guidelines and were authorized by local veterinary authorities.

Experiment. Compound 89 was administered to female C57Bl/6Nj mice. At indicated time points plasma and brain samples were quenched with organic solvent. (1 vol of sample + 3 vol of acetonitrile/methanol, 1:1 (v/v) containing an internal standard). After centrifugation for min at 4000g, the supernatant was diluted with one volume of aqueous 10 mM ammonium acetate at pH 3. Samples were then directly injected onto an LC-MS/MS system to quantify compound concentrations against a calibration curve using analyte specific transitions. Pharmacokinetic studies in rats were conducted accordingly using the following formulations: PO microsuspension in gelatin/NaCl (7.5%/0.62% in water) at 1.25 mg 89/mL; IV 0.5 mg 89/mL 0.9% NaCl in water/NMP (30/70).

Inhibition of A β 40 in Brain of Wild-Type Mice. Animals and Housing Conditions. Animals were maintained in a 12/12 h light/dark cycle, with lights starting at 6 a.m., and experiments were conducted during the light phase. Animal housing and experimental procedures were in line with ethical and legal guidelines and were authorized by local veterinary authorities.

Experiment. Female C57Bl/6j mice were treated with different doses of the compounds, 3–4 animals per treatment group. The test compound was dissolved in 5% EtOH, 10% Solurol, and was applied per os at 10 mL/kg. After 4 h, the animals were sacrificed and brain and plasma were collected. The brain was cut into halves and immediately frozen on dry ice. Brain was used for measurement of A β 40 and plasma was used for determination of compound exposure. The method for A β 40 determination in brain lysates followed the known procedure.⁶¹ Brain tissue was homogenized in 2% DEA buffer in a Roche MagnaLyser (20", 4000 rpm) and subsequently centrifuged for 1 h at 10000g. DEA was reduced to 0.2% in 50 mM NaCl and one-half of the DEA lysate was passed over an Oasis Solid phase extraction plate (Waters; cat. no. 186000679), which had been activated with MeOH and equilibrated in dH₂O (1 mL each). After washes in 10% and 30% MeOH (1 mL each), the A β -peptides were eluted in 0.8 mL of 2% NH₄OH in 90% MeOH. The eluate was dried over a N₂ flow, and the dried sample was reconstituted in 30 μ L of AlphaLISA assay buffer. A β 40 was determined by an AlphaLISA assay (Perkin-Elmer). In a white 96-well, half area microplate (Perkin-Elmer cat. no. 6005561), 20 μ L of the reconstituted sample were mixed with 5 μ L of biotinylated BAP-24 (specific for C-terminus of A β 40⁶²), stock = 4.4 mg/mL, i.e. 5.5 μ g/mL, and 5 μ L 252Q6 acceptor beads (252Q6 antibody, Invitrogen AMB0062) had been previously conjugated with AlphaLISA Acceptor beads (Perkin-Elmer cat. no. 6772002); final dilution 1:500. The mix was incubated for 1 h at RT in the dark. Then 20 μ L of Streptavidin-coated Donor Beads (Perkin-Elmer cat. no. 6760002, final dilution 1:125) were added and this final mix was incubated in the dark for another 30 min at RT before RFU was measured in an AlphaScreen Reader (Perkin-Elmer Envision 2104).

Inhibition of CSF A β 40 in Wild-Type Rats. Animals and Housing Conditions. Animals were maintained in a 12/12 h

light:dark cycle, with lights starting at 6 a.m., and experiments were conducted during the light phase. Animal housing and experimental procedures were in line with ethical and legal guidelines, and were authorized by local veterinary authorities.

Experiment. In this *in vivo* model, we followed the kinetics of changes in rat CSF A β 40 in an acute treatment study. For this purpose, male rats (Wistar) were prepared with a cannula inserted into the Cisterna magna.⁵⁷ Approximately 1 week after surgery, 2–3 animals were treated with one dose of the test compound by gavage (compound prepared in 10% solutol, 5% ethanol, and 85% water, 4 mL/kg). CSF was drawn at 2 and 1 h before compound application and then at different time points postapplication, the volume of CSF withdrawn at each time-point was about 10 μ L. For the determination of A β -peptide in the rat CSF a commercial ELISA kit specific for A β 40 was used according to the protocol of the manufacturer (WAKO, cat. no. 294–64701, human/rat β -amyloid 40). Because of interanimal differences in baseline A β 40 level in CSF the pretreatment samples were used to define the 100% A β 40 level for each individual animal and the subsequent post-treatment values were expressed as percentage of this control value.

■ ASSOCIATED CONTENT

■ Supporting Information

Preparation details and analytical data for compounds 1a–b, 14b–c, 14e–v, 66–70, 88, 109–113. Log *D*, p*K*_i determinations, stability in mouse microsomes, inhibition of CYP₄₅₀, and time dependent inhibition of CYP₄₅₀ 3A4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Accession Codes

Crystal structure coordinates and structure factors have been deposited to the PDB with the accession numbers 4JOP (1b), 3ZMG (14d), 4JOT (14i), 4JOV (66), 4JOY (67), 4JOZ (68), 4J17 (69), 4J1C (70), 4J1E (88), 4J1F (89), 4J1H (109), 4J1I (110), and 4J1K (111). The structure of BACE2 in complex with 14i has been deposited with the accession number 3ZLQ.

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Notes

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■ ABBREVIATIONS USED

HERG, human ether-à-go-go related gene; DCM, dichloromethane; Deoxo-Fluor, bis(2-methoxyethyl)aminosulfur trifluoride; DIBALH, diisobutylaluminumhydride; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; DMTMM, 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholinium chloride hydrate; EtOAc, ethyl acetate; EtOH, ethanol; MeOH, methanol; TBME, *tert*-butylmethylether; TEA, triethylamine; TFA, trifluoro-acetic acid; T3P, (2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide); TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran

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F. Hoffmann - la Roche AG y
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Exp. 13-250.072 IV)

NOTICE OF ALLOWANCE AND FEE(S) DUE

112867 7590 03/12/2014
Hoffmann-La Roche Inc.
C/O Gibbons P.C.
One Gateway Center
Newark, NJ 07102

EXAMINER

HABTE, KAHSAY

ART UNIT

PAPER NUMBER

1624

DATE MAILED: 03/12/2014

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/467,085	05/09/2012	Hans Hilpert	111890-83098 (27315 US)	5486

TITLE OF INVENTION: 1,3-OXAZINES AS BACE 1 AND/OR BACE2 INHIBITORS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$960	\$0	\$0	\$960	06/12/2014

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. **PROSECUTION ON THE MERITS IS CLOSED.** THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. **THIS STATUTORY PERIOD CANNOT BE EXTENDED.** SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or **Fax** (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

112867 7590 03/12/2014
Hoffmann-La Roche Inc.
C/O Gibbons P.C.
One Gateway Center
Newark, NJ 07102

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/467,085	05/09/2012	Hans Hilpert	111890-83098 (27315 US)	5486

TITLE OF INVENTION: 1,3-OXAZINES AS BACE 1 AND/OR BACE2 INHIBITORS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$960	\$0	\$0	\$960	06/12/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
HABTE, KAHSAI	1624	514-228800

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/467,085	05/09/2012	Hans Hilpert	111890-83098 (27315 US)	5486
112867	7590	03/12/2014	EXAMINER	
Hoffmann-La Roche Inc. C/O Gibbons P.C. One Gateway Center Newark, NJ 07102			HABTE, KAHSAI	
			ART UNIT	PAPER NUMBER
			1624	

DATE MAILED: 03/12/2014

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 189 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 189 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.** Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/467,085	Applicant(s) HILPERT ET AL.	
	Examiner KAHSAY HABTE	Art Unit 1624	AIA (First Inventor to File) Status No

- The MAILING DATE of this communication appears on the cover sheet with the correspondence address-

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the Initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 2/27/2014.
☐ A declaration(s)/affidavit(s) under 37 CFR 1.130(b) was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 1-27. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☒ All b) ☐ Some *c) ☐ None of the:

1. ☒ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. _____.

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has **THREE MONTHS FROM THE "MAILING DATE"** of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in **ABANDONMENT** of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ **CORRECTED DRAWINGS** (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ **DEPOSIT OF and/or INFORMATION** about the deposit of **BIOLOGICAL MATERIAL** must be submitted. Note the attached Examiner's comment regarding **REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL**.

Attachment(s)

1. ☒ Notice of References Cited (PTO-892)	5. ☐ Examiner's Amendment/Comment
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date <u>7/9/12 & 6/29/12</u>	6. ☒ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material	7. ☐ Other _____
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____	

DETAILED ACTION

1. Claims 1-27 are pending in this application.

Election/Restrictions

2. The Restriction Requirement dated 01/14/2014 is withdrawn.

Reasons for Allowance

3. The following is an examiner's statement of reasons for allowance: claims 1-27 are free from prior art. The closest prior art is Zhu et al. *Organic Letters* (2006), 8(12), 2599-2602 that discloses 4H-1,3-Oxazin-2-amine, 5,6-dihydro-4-(4-methylphenyl)-6-phenyl-, hydrochloride compound that is almost the same as applicants except in said prior art the 6-position is substituted by phenyl instead of hydrogen or alkyl.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Information Disclosure Statement

4. Applicant's Information Disclosure Statement, filed on 07/09/2012 and 06/29/2012 has been acknowledged. Please refer to Applicant's copies of the 1449 submitted herewith.

Conclusion

5. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kahsay Habte Ph.D. whose telephone number is (571) 272-0667. The examiner can normally be reached on M-F (7:00 AM- 3:30PM).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James O. Wilson can be reached at (571) 272-0661. The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Kahsay Habte/
Primary Examiner, Art Unit 1624

March 7, 2014

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-03 CAMBIO DE MODALIDAD Y MODIFICACIONES	EN N. CREACION CORRECCION A SOLICITUD EN TRAMITE / MODIFICA	132.000.00	132.000.00
				\$132.000.00

SON: **CIENTO TREINTAY DOS MIL PESOS MONEDA CORRIENTE**

Responsable: _____

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-258872-00006-0000

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