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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Trámite: 11
Evento: 378
Actuación: 412

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 BACE1 Y/O BACE2".-Clase C07D 265/08.-Expediente
número 13-258.872-

Yo, **ALICIA LLOREDA RICAURTE**, mayor, vecina de esta ciudad, identificada con la cédula de ciudadanía número 39.690.713 de Usaquén, Representante Legal en Bogotá para todos los asuntos relacionados con la Propiedad Industrial de la sociedad **F.HOFFMANN-LA ROCHE AG**, organizada y existente de conformidad con las leyes De Suiza y domiciliada en Basilea Suiza y la sociedad **SIENA BIOTECH**, organizada y existente con las leyes de Italia y domiciliada en Siena, Italia, atentamente manifiesto a usted que interpongo **RECURSO DE REPOSICIÓN** contra la Resolución Número **28648** dictada por el señor Superintendente de Industria y Comercio el 29 de Mayo de 2015 y notificada por fijación en lista el 2 de Junio de 2015, por medio de la cual se niega el privilegio de patente solicitado para la invención contenida en el expediente de la referencia.

I. FUNDAMENTOS DE DERECHO

Como fundamento de mi recurso, me permito manifestar ante ese Despacho que el contenido de la Resolución Número **28648** evidencia que durante el examen técnico realizado a la solicitud denegada se realizó una evaluación incorrecta del objeto de la invención allí reclamada, sin considerarse su real alcance e importancia. En este sentido, no se consideró el verdadero y valioso aporte técnico que representa para el arte en cuestión los novedosos compuestos derivados de [1,3]-oxazinas con actividad inhibitoria de la BACE1 y/o BACE2, revelados en la solicitud denegada.

Es así que, a pesar de los argumentos proporcionados por el solicitante en respuesta al Concepto Técnico correspondiente al Oficio No. 14551 y notificado por fijación en lista por ese Despacho el 25 de Noviembre de 2014, en la Resolución impugnada este Despacho mantiene las objeciones frente al Nivel Inventivo de la solicitud, argumentando básicamente que:

"(...) una persona normalmente versada en la materia fácilmente esperaría se conservara el efecto terapéutico al reemplazar el anillo dihidrotiazina divulgado en el documento D2 por un anillo oxazina dada la relación bioisostérica existente entre los grupos -O- y -S-, manteniendo el grupo amino sustituyendo la posición 2 y los grupos X y Z enlazados por grupos -NHCH2- o -NH- como se sugiere en el documento D2 para llegar así al objeto de la reivindicación 5 de la solicitud".

Además, el señor Examinador aclara que:

"(...) la solicitante no presenta evidencia del efecto técnico sorprendente presentado por los compuestos 1,3 oxazinas reclamados con respecto a los compuestos dihidrotiazina conocidos en el estado de la técnica. Lo anterior teniendo en cuenta que la solicitud establece que para los compuestos reclamados se determinaron valores IC50 para BACE1 que varían entre 0,01 y 15 μ M (Ver págs. 77 a 88 de la descripción; radicado N° 13-258872-00000-0000). El documento D2 establece valores IC50 menores a 1 μ M, por lo que se puede observar que estos valores se encuentran dentro del rango establecido para los compuestos de la invención."

Igualmente, el señor Examinador recalca que:

"(...) el documento D3 proporciona la motivación suficiente para reemplazar un anillo tiazina por un anillo oxazina dada la relación bioisostérica existente y ampliamente conocida entre los átomos de oxígeno y azufre como una diversas opciones igualmente probables dentro de un diseño experimental rutinario para obtener compuestos alternativos con respecto a los compuestos divulgados en el compuesto D2 y que mantengan el efecto inhibidor de las enzimas BACE1 y BACE2, más aún cuando la solicitud no demostró a largo del trámite un efecto sorprendente o mejorado de los compuestos reclamados los conocidos en el estado de la técnica".

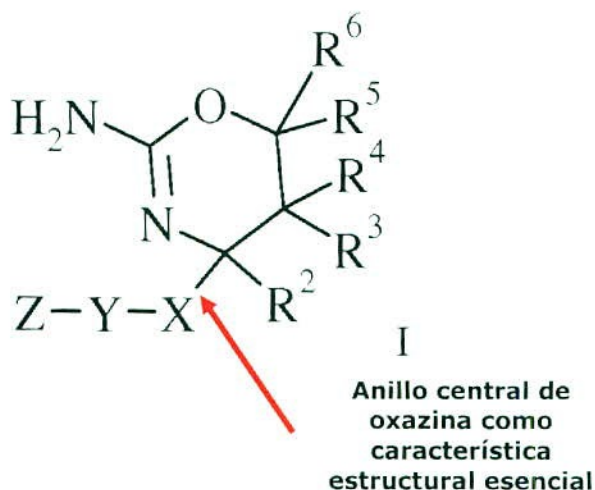
Frente a las anteriores consideraciones, a continuación me permito demostrar ante ese Despacho que la presente invención sí cumple a cabalidad con el requisito de nivel inventivo exigido por el artículo 18 de la Decisión 486 de 2000, toda vez que los compuestos de fórmula (I), derivados de compuestos derivados de [1,3]-oxazinas con actividad inhibitoria de la BACE1 y/o BACE2 de ninguna manera habrían podido ser

predichos u obtenidos de manera evidente por la persona medianamente versada en la materia a partir de las enseñanzas del estado de la técnica anterior.

1. PRINCIPALES CARACTERÍSTICAS DE LA INVENCION DENEGADA.

Con el fin de contextualizar los motivos por los cuales ese Despacho continúa realizando una incorrecta valoración y evaluación del arte previo, me permito resaltar las principales características y ventajas técnicas asociadas a la materia revelada en la solicitud de patente negada.

En vista del capítulo reivindicatorio actualmente presentado, la presente solicitud reclama de manera específica y exclusiva compuestos orgánicos derivados de 5,6-dihidro-4H-[1,3]oxazin-2-ilaminas, que tienen propiedades inhibitoras de la BACE1 y/o de la BACE2 y que cuentan con la siguiente fórmula (I):



Con relación a las características esenciales de los compuestos reclamados, me permito resaltar ante ese Despacho que su caracterización cumple con los requerimientos necesarios para definir las características técnicas de un compuesto químico. En este sentido, la reivindicación 1 actualmente presentada presenta las definiciones específicas para los grupos funcionales comprendidos en cada uno de los sustituyentes de la anterior fórmula, y por lo tanto presenta claramente las características técnicas estructurales de los compuestos reclamados. Adicionalmente, el capítulo reivindicatorio reclama compuestos específicos que cumplen con las restricciones establecidas en las reivindicaciones precedentes.

Ahora, con respecto a las ventajas y beneficios sorprendentes de la presente invención, me permito traer a colación que los compuestos de la presente son útiles para el control o la prevención de enfermedades, por ejemplo la enfermedad de Alzheimer y la diabetes de tipo 2, ya que los nuevos compuestos de la fórmula I tienen propiedades farmacológicas mejoradas y valiosas.

Específicamente, los presentes compuestos de la invención tienen actividad inhibidora de la Asp2 (β -secretasa, BACE1 o memapsina-2) y por esta razón pueden utilizarse para el tratamiento terapéutico y/o profiláctico de enfermedades y trastornos caracterizados por niveles elevados de β -amiloides y/u oligómeros de β -amiloides y/o placas de β -amiloides y depósitos diversos, en especial la enfermedad de Alzheimer. Adicionalmente, los presentes compuestos de fórmula I tienen actividad inhibidora de la BACE2 y por ello pueden utilizarse para el tratamiento terapéutico y/o profiláctico de enfermedades y trastornos, tales como la diabetes de tipo 2 y otros trastornos metabólicos.

Cabe resaltar que los compuestos presentes inhiben además la formación o la formación y la deposición de las placas de β -amiloides en, sobre o alrededor del tejido neurológico (p.ej. en el cerebro), ya que inhiben la producción de $A\beta$ a partir de la APP o de un fragmento de la APP.

En particular, la Tabla de las páginas 77 a 88 de la descripción de la presente solicitud proporciona los valores IC50 para BACE 1 y BACE2 que demuestran que los compuestos de la presente invención inhiben satisfactoriamente la actividad de dichas enzimas. Concretamente, y de acuerdo a lo que señala el señor Examinador en la Resolución aquí impugnada, los compuestos reclamaron exhiben valores IC50 para BACE1 que varían entre 0.011 μ M (*compuesto del ejemplo 22*) y 14.466 μ M (*compuesto del ejemplo 51*). A su vez, 20 compuestos de los 66 reclamados exhiben valores IC50 para BACE que varían entre 0.068 μ M (*compuesto del ejemplo 14*) y 10.861 μ M (*compuesto del ejemplo 40*). Es importante aclarar que dicha actividad inhibitoria es exclusivamente atribuida a compuestos derivados de oxazinas.

A la luz de las anteriores consideraciones, me permito concluir ante ese Despacho que los compuestos derivados de [1,3]-oxazinas constituyen novedosas moléculas con una actividad inhibitoria para las enzimas BACE1 y/o BACE2, lo que los convierte en potenciales componentes de productos medicinales y terapéuticos. Dichas características técnicas esenciales de los particulares compuestos se evidencian a lo largo de la descripción de la solicitud, particularmente en los ejemplos realizados.

En consecuencia, es evidente que los compuestos reivindicados en la presente solicitud tienen una particularidad estructural que deriva en un efecto sorprendente e inesperado con respecto al estado del arte.

2. CONSIDERACIONES TÉCNICAS FRENTE A LAS ENSEÑANZAS DEL ARTE PREVIO

Ahora, habiendo resaltado el alcance del capítulo reivindicatorio actualmente presentado de la invención en cuestión, me permito manifestar ante ese Despacho que los

compuestos de fórmula I, derivados de [1,3]-oxazinas, en la solicitud de patente denegada, presentan un inequívoco nivel inventivo frente 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 la Resolución aquí impugnada, el señor Examinador considera que la composición reclamada en la solicitud no tiene nivel inventivo puesto que, a la luz de las enseñanzas de los documentos D2 y D3, la persona del oficio normalmente versada en la materia podría derivar de manera evidente el objeto de la reivindicación 1 de la solicitud.

En relación con lo anterior, **me permito sostener que las enseñanzas de dichos documentos, solas o en combinación, de ninguna manera anticipan o sugieren de manera obvia los compuestos derivados de [1,3]-oxazinas de fórmula I con todas y cada una de las definiciones específicas para sus sustituyentes y que a su vez conserven su específica actividad inhibitoria, tal y como se reclaman en el capítulo reivindicatorio actualmente presentado.**

Con el ánimo de demostrar ante ese Despacho que la anterior afirmación es completamente correcta, a continuación me permito resaltar el análisis comparativo ya establecido entre la información revelada en la presente solicitud y las enseñanzas brindadas por dichas anterioridades.

2.1. Las diferencias estructurales existentes entre los compuestos de la presente invención y aquellos divulgados en el documento D2 no son evidentes para la persona medianamente versada en la materia.

De acuerdo a los argumentos proporcionados por el solicitante en respuesta al Concepto Técnico correspondiente al Oficio No. 14551 y notificado por fijación en lista por ese Despacho el 25 de Noviembre de 2014, me permito resaltar nuevamente que 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 mientras que los compuestos divulgados en D2 presentan como parte fundamental de su molécula un grupo tiazinilo, y
- ii) los compuestos de la invención no definen ningún grupo carbonilo para el sustituyente Z mientras que todos los compuestos divulgados en D2 incluyen dicho grupo carbonilo unido a un grupo amino, creando así una amida en la estructura y estableciendo que dichos compuestos son derivados de la amida del ácido carboxílico sustituido.

Lo anterior implica 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: a) tendría que hacer el cambio del átomo de azufre por el átomo de oxígeno en el anillo central de la dihidrotiazina, y b) eliminar el grupo carbonilo para la posición correspondiente al sustituyente Z. Es importante aclarar que, para poder realizar dichos cambios en D2, las enseñanzas del documento D3 deberían usarse como motivación fundamental.

Sin embargo, el documento D3 relaciona conceptos sobre bioisosterismo y omite cualquier referencia a algún compuesto específico relacionado con el campo de la invención. Específicamente, divulga únicamente el reemplazo bioisostérico entre los grupos -S- y -O-, pero no especifica que dicho reemplazo es satisfactorio en fracciones de anillos heterocíclicos ni tampoco menciona la eliminación de un grupo carbonilo en la posición del sustituyente Z de la fórmula I de los compuestos de la presente invención.

Consecuentemente, **me permito señalar que el documento D3 no brinda ninguna sugerencia acerca de que las modificaciones que deben realizarse en los compuestos de D2 con el fin de llegar a obtener los compuestos específicos reclamados en la solicitud denegada.**

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.

2.2. Respuesta a los argumentos presentados en la Resolución impugnada.

De conformidad con los anteriores argumentos, me permito señalar ante ese Despacho que las afirmaciones presentadas en la Resolución impugnada carecen de toda relevancia para desvirtuar la novedad y el nivel inventivo de la presente solicitud, tal como me permito señalar a continuación:

- ✓ En la Resolución Número **28648** de la SIC se aclara que "(...) si bien los compuestos ejemplificados en el documento D2 corresponden a compuestos derivados de la amida de un ácido carboxílico (incluyendo los compuestos de los Ejemplos 655, 669, 674, 468 y 551, de los cuales argumenta la solicitante corresponden a los compuestos con mayor potencia inhibitoria para BACE1 divulgados en el documento D2), la definición de la formula Markush divulgada

en el documento D2 establece que el anillo A puede corresponder a un grupo B enlazando a un sustituyente N(R5) donde B es un anillo carbocíclico opcionalmente sustituido o heterocíclico opcionalmente sustituido (Pág. 248, líneas 18 y 19) y R5 es hidrógeno, alquilo inferior o acilo (Pág. 248, línea 11), lo cual es una motivación a utilizar el sustituyente -NH- (cuando el sustituyente R5 es hidrógeno), como sustituyente Y, en lugar del sustituyente carboxamida (...)".

En respuesta a lo anterior, y de conformidad con lo mencionado a lo largo del presente recurso, atentamente me permito señalar una vez más ante ese Despacho lo siguiente:

Efectivamente, el documento D2 reivindica la posibilidad de utilizar el sustituyente -NH- en la correspondiente posición, pero dicha sugerencia no se encuentra en ninguna parte del documento D3. Frente a esto, me permito nuevamente resaltar que 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 por las enseñanzas generales del documento D3, sino que si lo hubiera hecho de esa manera, ¿por qué dichas enseñanzas del documento D3 lo motivaron a hacerlo con la expectativa de resolver el problema técnico objetivo?

Como ya se explicó anteriormente, no existe información alguna en D3 para que, del gran número de posibilidades resultantes con las restricciones del documento D2, la persona versada en la materia pudiese escoger específicamente dicha limitación al sustituyente -NH- y obtener, de manera evidente, compuestos con el efecto técnico atribuido a la invención. Esencialmente, la cuestión no es obtener la información fraccionada del mismo D2 sino motivarse exclusivamente de las enseñanzas de D3 para poder modificar compuestos de D2, dentro de las limitaciones establecidas, y así alcanzar el objeto de la invención.

Frente lo anterior, muy respetuosamente me permito manifestar que el señor examinador hace un incorrecto análisis del nivel inventivo. Así las cosas, es claro que la persona versada en la materia no podría seleccionar dichas modificaciones al azar, teniendo en cuenta que D3 únicamente discute el posible cambio bioisostérico entre oxígeno y azufre sin mencionar alguna posible eliminación de la carboxamida. Es más, dicha persona versada en la materia tampoco podría derivar de manera evidente los compuestos de la invención dado que las enseñanzas de D3, combinadas con D2, no aseguran que posibles modificaciones resulten en compuestos con la misma actividad inhibitoria satisfactoria de BACE1 y/o BACE2.

- ✓ De otro lado, en la Resolución impugnada se afirma que "(...) *no es claro cuando la solicitante afirma que "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", puesto que de acuerdo a la tabla 1 se puede observar que las tiazinas presentan mayor actividad como inhibidores de BACE1, al presentar menores valores de IC50 (μM) para la enzima BACE1, lo cual en ningún caso puede llevar a concluir que para la persona normalmente versada en la materia no sería obvio el reemplazo de un anillo tiazina por un anillo oxazina, al presentarse una menor actividad como inhibidor de BACE1 y BACE2 por parte de las oxazinas, puesto que este documento fue publicado con fecha de 16 de abril de 2013 y por tanto de ninguna manera podía corresponder a un documento de conocimiento público el día 16 de mayo de 2011 (fecha de prioridad invocada) (...)"*.

Frente a éste particular, en primer lugar me permito manifestar que el documento referente al estudio realizado por Hilpert et al., que se adjunta al presente memorial, debe ser considerado –a pesar de su fecha de publicación– porque 1) el artículo es útil para evidenciar el hecho que el análisis del señor Examinador sobre el remplazo convencional del grupo tiazinilo por el grupo oxazinilo y/o el remplazo de hidrógeno por flúor es incorrecto y 2) este artículo no es utilizado para evaluar los requerimientos de novedad o nivel inventivo de la presente solicitud. En realidad, dicho artículo respalda la idea que el remplazo de azufre por oxígeno en la correspondiente posición del anillo no es obvio para este caso.

Ahora, tal y como lo manifiesta el señor Examinador en su recurso, las tiazinas presentan mayor actividad como inhibidores de BACE1, al presentar menores valores de IC50 (μM) para la enzima BACE1. En este sentido, no se esperaría que la persona versada en la materia se viera motivada a cambiar un átomo de azufre por uno de oxígeno con el fin de obtener un anillo de oxazina, máxime si se soporta la idea que las oxazinas tienen menor actividad inhibitoria. En palabras más sencillas, ¿qué motivaría a la persona versada en la materia a utilizar oxazinas para inhibir BACE1 y/o BACE2, si la literatura sugiere que los mejores compuestos para tal inhibición son las tiazinas?

Al leer los resultados de la Tabla 1 y la sección de "Resultados y Discusión" de dicho artículo, se puede concluir el hecho científico de que el remplazo del grupo tiazinilo por el grupo oxazinilo en actividades de hBACE1 y hBACE2, los ensayos celulares y la eficacia *in vivo* son muy complicados e involucran cambios extraordinarios (*la actividad inhibitorio es menor*). Consecuentemente, **dicho artículo claramente evidencia que el remplazo de un anillo de tiazina por uno de oxazina no son cambios ni remplazos convencionales o de rutina para una persona versada en la materia.**

- ✓ Finalmente, en la Resolución impugnada se concluye que "(...) el documento D3 proporciona la motivación suficiente para reemplazar un anillo tiazina por un anillo oxazina dada la relación bioisostérica existente y ampliamente conocida entre los átomos de oxígeno y azufre como una diversas opciones igualmente probables dentro de un diseño experimental rutinario para obtener compuestos alternativos con respecto a los compuestos divulgados en el compuesto D2 y que mantengan el efecto inhibidor de las enzimas BACE1 y BACE2, más aún cuando la solicitud no demostró a largo del trámite un efecto sorprendente o mejorado de los compuestos reclamados los conocidos en el estado de la técnica (...)".

Frente a tal consideración, me permito nuevamente señalar que, como se ha venido explicando, el remplazo 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.

Si bien compuestos el intercambio isostérico entre los átomos de azufre y oxígeno es conocido en la materia, dichas modificaciones representan una posible alteración en una variedad de parámetros moleculares. Reiteradamente, me permito señalar que **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 remplazos 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 remplazos 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 señala Thornber²: *"al hacer un remplazo bioisostérico, los siguientes parámetros del*

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

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

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 otras palabras, la alteración a partir de las modificaciones isostéricas se refleja específicamente en³:

- ✓ Parámetros estructurales: cuando el cambio isostérico contribuye a una orientación geométrica diferente.
- ✓ Parámetros electrónicos: cuando el cambio isostérico participa en interacciones eléctricas que pueden afectar el pK_a y el grado de ionización.
- ✓ Propiedades farmacocinéticas: cuando el cambio isostérico participa en la absorción, distribución y/o excreción de la molécula.

En este sentido, y teniendo en cuenta que la literatura sugiere que anillos de oxazina exhibirían una menor inhibición de BACE1 y/o BACE2 y que los reemplazos bioisostéricos acarrearán cambios relevantes en la actividad fisicoquímica del compuesto, es sorprendente e inesperado que los compuestos de la presente invención exhiban una actividad inhibitoria satisfactoria en comparación con los compuestos del estado del arte previo. Consecuentemente, me permito afirmar que, contrario a lo que asegura el señor Examinador, los compuestos de la invención **sí** demuestran un efecto técnico sorprendente e inesperado dado que son oxazinas con actividad inhibitoria de la BACE1 y/o BACE2.

Ahora, frente al solapamiento de los valores IC50 de los compuestos de la invención con respecto a aquellos de D2, me permito resaltar que no es correcto comparar dichos valores puesto que los compuestos corresponden a estructuras con anillos centrales diferentes. Para afirmar que los valores son ya revelados, es necesario comparar compuestos con la misma naturaleza; es decir, que los compuestos de la invención exhibieran un anillo de tiazina en el núcleo central. Sin embargo, los compuestos reclamados son derivados de oxazinas, los cuales presentan una actividad inhibitoria sorprendente. En realidad, **en ninguna parte de los documentos de la anterioridad se presentan compuestos derivados de [1,3]oxazinas con los mismo valores de IC50, referentes a la inhibición de BACE1 y/o BACE2.**

³ Facultad de Ciencias Bioquímicas y farmacéuticas, Universidad Nacional de Rosario, Argentina. "Variaciones basadas en reemplazos bioisostéricos".

Para concluir, me permito de nuevo preguntar, ¿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, a partir de las enseñanzas del documento D3, 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 nuevamente 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 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 D3 no pueden de ninguna manera afectar el Nivel Inventivo de la solicitud en estudio.

3. CONCESIÓN DE LA PRESENTE SOLICITUD POR PARTE DE OTRAS OFICINAS DE PATENTES.

Finalmente y retomando el contenido de la respuesta al Concepto Técnico correspondiente al Oficio No. 14551 y notificado por fijación en lista por ese Despacho el 25 de Noviembre de 2014, atentamente me permito resaltar ante ese Despacho que la materia revelada en la solicitud de patente en cuestión, ha sido considerada como materia patentable por parte de los examinadores de la Oficina Americana de Patentes, USPTO, y como consecuencia de ello ha sido concedida la patente US 8,785,436 (Anexos iii) y iv) del presente recurso corresponden a una copia de la primera página del documento de dicha

patente americana con Número de Publicación No. US 2012/295900 y copia de la noticia de su concesión– Notice Of Allowance, respectivamente).

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, considero de gran importancia resaltar el hecho particular de que la patentabilidad de la solicitud ha sido reconocida por los Examinadores de la USPTO, teniendo en cuenta, lo establecido en la Resolución Número 54093 de 2012, la cual en su numeral 1.2.2.5.21, establece el "*Reconocimiento de exámenes de patentabilidad de solicitudes extranjeras*", y de donde se desprende la posibilidad de que, a pesar de la independencia con la que cuenta la SIC, ese Despacho ha empezado a darle credibilidad a las decisiones tomadas por las autoridades de Patentes de otros países, a través de la figura del "Patent Prosecution Highway PPH".

En este sentido, Colombia ha firmado el tratado del PPH con Estados Unidos, permitiendo así reconocer que a pesar de la independencia de las Oficinas de Patentes, si una solicitud ha sido reconocida como patentable en dicho país como en el presente caso Estados Unidos, se entiende que la decisión emitida por dicha oficina, tiene más peso y puede ser tenida en cuenta a la hora de revisar la patentabilidad de una solicitud.

Por lo anterior, muy respetuosamente solicitamos a ese Despacho, que a la hora de revisar este caso, tenga en cuenta el resultado emitido por otra Autoridad de Patente, como lo es la USPTO, y que el mismo sea considerado como un indicio fuerte de que la presente solicitud sí cuenta con los requisitos de patentabilidad exigidos por la normativa andina.

II. CONCLUSIÓN

Con base en los argumentos técnicos mostrados previamente me permito manifestar ante ese Despacho que el capítulo reivindicatorio actualmente presentado goza de un inequívoco carácter inventivo frente a las enseñanzas de los documentos D2 y D3, razón por la cual dicha materia es clara merecedora del privilegio de patente solicitado.

III. PETICIÓN

En vista de todo lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las argumentaciones presentadas, y proceder a revocar la Resolución

Número **28648**, con el fin de que se conceda el privilegio de patente solicitado para la presente invención.

IV. PODER

En este punto me permito mencionar ante ese Despacho, que los documentos de poder, que acredita mi calidad de Representante Legal en Bogotá para asuntos relativos a la Propiedad Industrial de las sociedades peticionarias, fueron presentados junto con la solicitud de patente el 31 de octubre de 2013.

V. ANEXOS

Anexo al presente recurso me permito presentar:

- i) 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;
- ii) Copia del documento citado "Variaciones basadas en reemplazos bioisostéricos", Facultad de Ciencias Bioquímicas y farmacéuticas, Universidad Nacional de Rosario, Argentina;
- iii) Copia de la Carátula y capítulo reivindicatorio del documento de la patente americana US 8,785,436 (Pub. No. 2012/295900), y
- iv) Copia de la decisión de conceder la patente 8,785,436 por parte de la USPTO.

VI. DOMICILIO

Mi dirección es Calle 72 No. 5-83, Piso 6º, Bogotá D.C.

Del Señor Superintendente,


ALICIA LLOREDA RICAURTE

T.P. 53.215

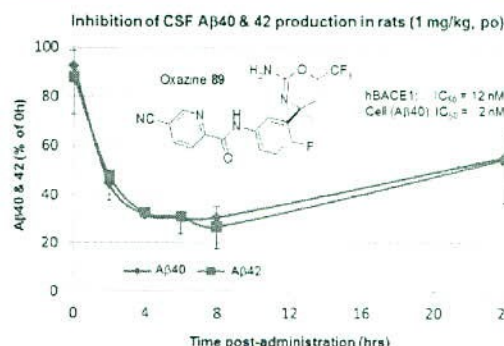
β -Secretase (BACE1) Inhibitors with High in Vivo Efficacy Suitable for Clinical Evaluation in Alzheimer's Disease

Hans Hilpert,^{*,†} Wolfgang Guba,[†] Thomas J. Woltering,[†] Wolfgang Wostl,[†] Emmanuel Pinard,[†] Harald Mauser,[†] Alexander V. Mayweg,[†] Mark Rogers-Evans,[†] Roland Humm,[†] Daniela Krummenacher,[†] Thorsten Muser,[†] Christian Schnider,[†] Helmut Jacobsen,[‡] Laurence Ozmen,[‡] Alessandra Bergadano,[‡] David W. Banner,[§] Remo Hochstrasser,[§] Andreas Kuglstatte,[§] Pascale David-Pierson,^{||} Holger Fischer,^{||} Alessandra Polara,[⊥] and Robert Narquizian[⊥]

[†]Discovery Chemistry, [‡]DTA Neuroscience, [§]Discovery Technologies, ^{||}Drug Metabolism and Pharmacokinetics, [⊥]Roche Partnering, F. Hoffmann–La Roche Ltd, pRED, Pharma Research & Early Development, Grenzacherstrasse 124, Basel CH-4070, Switzerland

S 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-4*H*-5,6-dihydro-[1,3]thiazine derivative **A** (Figure 1) which could be cocrystallized with

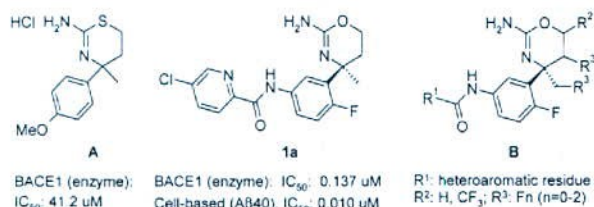


Figure 1. Lead compound **1a** and targeted fluoro-oxazines **B**.

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-4*H*-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.

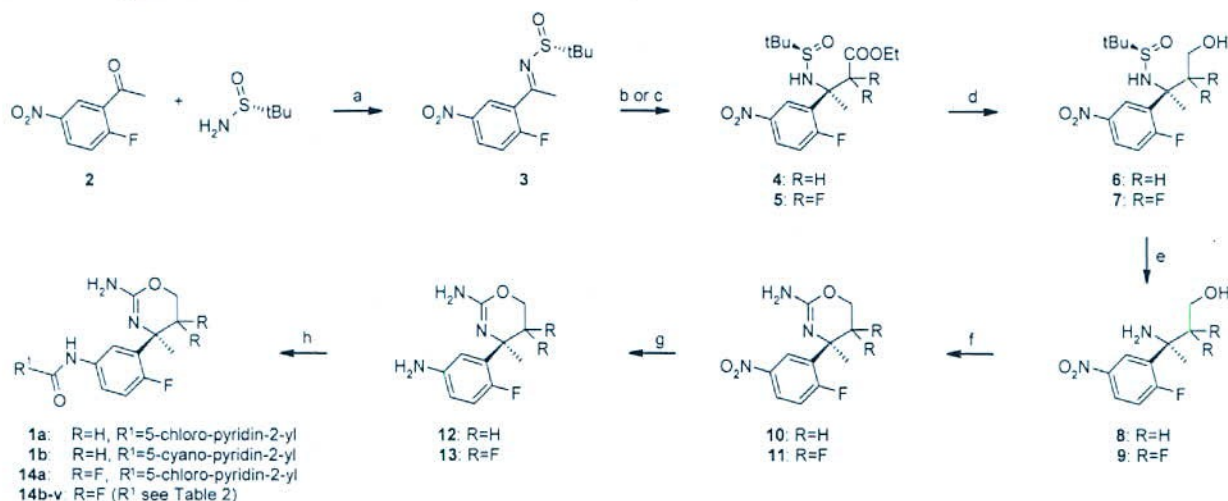
CHEMISTRY

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**.

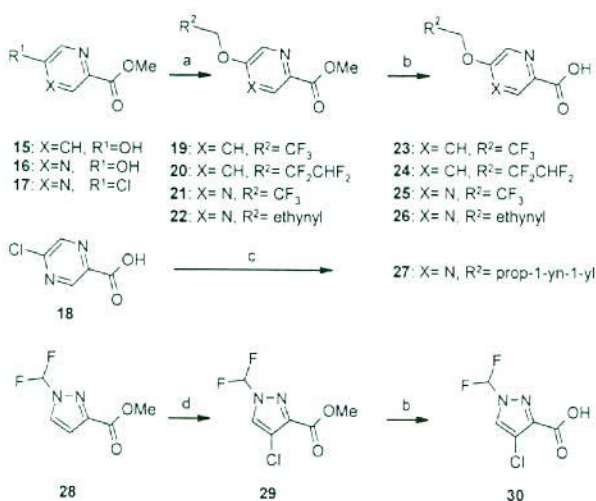
The ester group in **4** and **5** was then reduced to the alcohols **6** and **7** with LiBH₄, which was followed by cleavage of the chiral auxiliary under acidic conditions to give the amino alcohols **8** and **9**.³² Cyclization of the amino alcohols **8** and **9** was readily accomplished with cyanogen bromide to give the oxazines **10** and **11**, which were reduced to the corresponding anilines **12**³³ 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

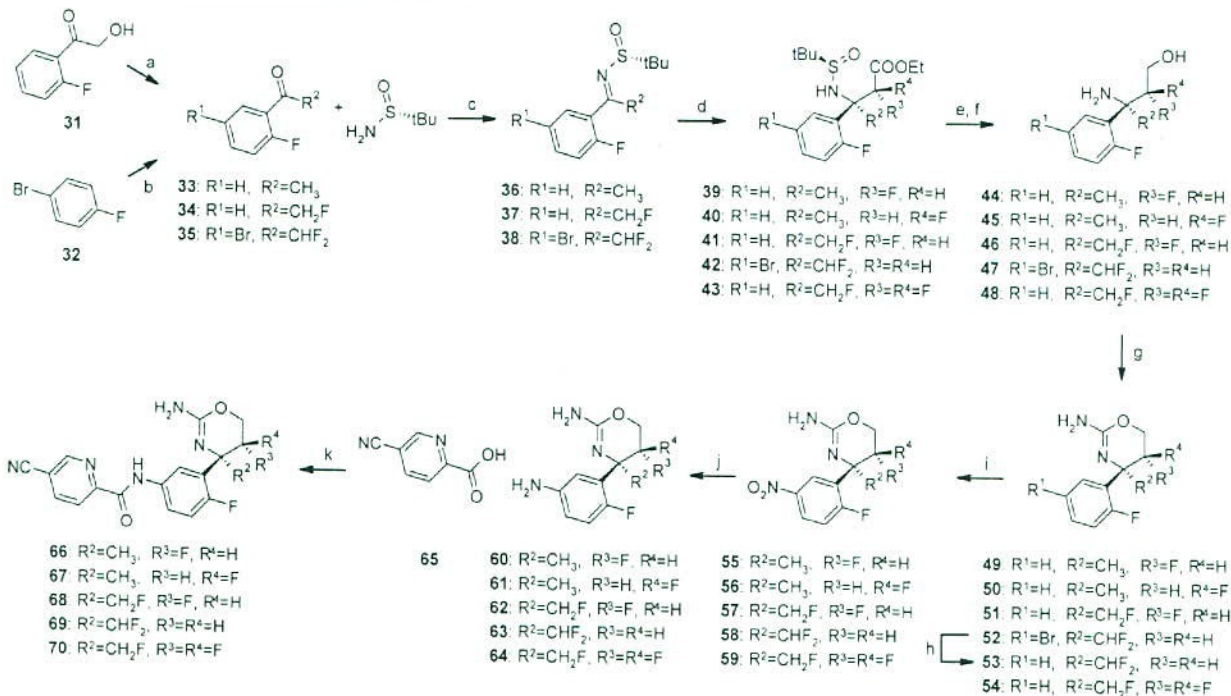
^aReagents and conditions: (a) 15→19: CF₃CH₂OTf, NaH, DMF, 22 °C. 15→20: CHF₂CH₂CH₂OTf, K₂CO₃, acetone. 17→21: CF₃CH₂OH, Cs₂CO₃, DMF. 17→22: prop-2-yn-1-ol, *tert*-BuOK, 1,4-dioxane, 22 °C. (b) LiOH or NaOH, THF/H₂O, 22 °C to reflux. (c) But-2-yn-1-ol, *tert*-BuOK, DMF, 65 °C. (d) *N*-chlorosuccinimide, 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).

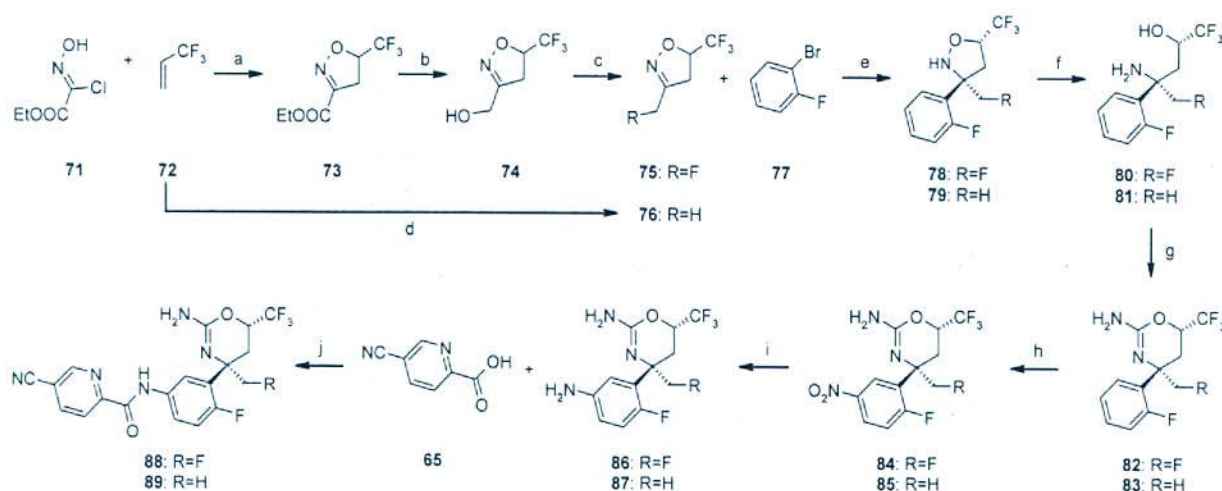
Compound 27 was prepared directly from the acid 18 without proceeding via the corresponding ester. Finally, chlorination of the pyrazole ester 28 with *N*-chlorosuccinimide followed by saponification delivered the chloro-pyrazole derivative 30.

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 difluoroacetate. 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 BrCH₂COOEt yielded a separable 4:1-mixture of the epimers 39 and 40. Conversion of 37 with BrCH₂COOEt 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.

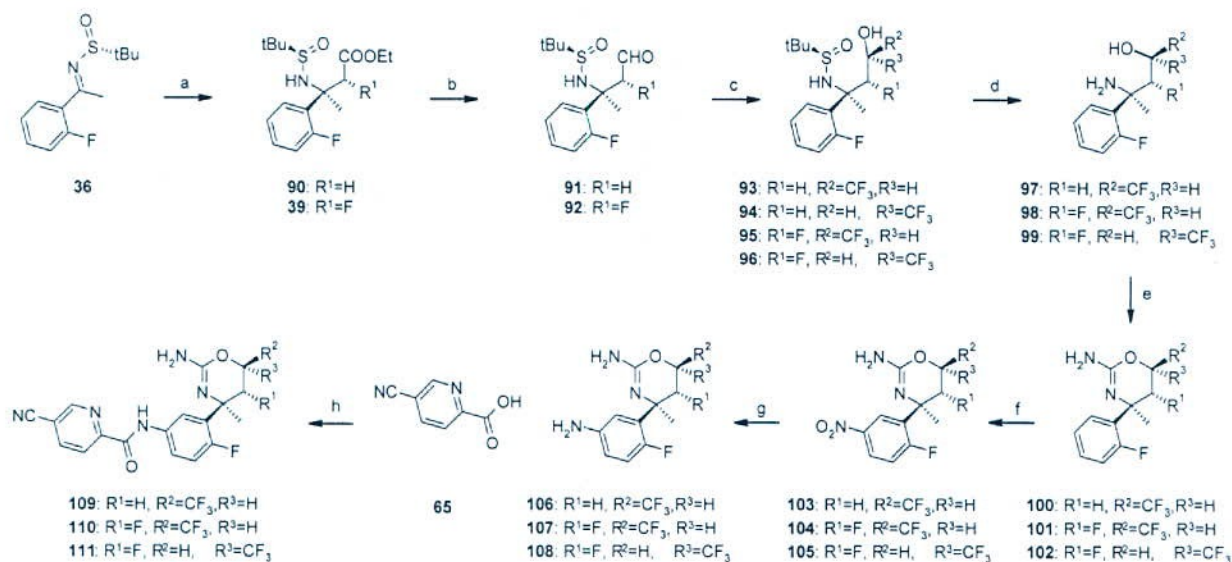
The synthesis of trifluoromethyl substituted oxazines 88 and 89 (Scheme 4) required a different strategy. Ready access to CF₃ substituted 3-amino-propan-1-ol derivatives 80 and 81 is achieved using a dipolar cycloaddition reaction of a nitrile oxide

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: BrCH₂COOEt, 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

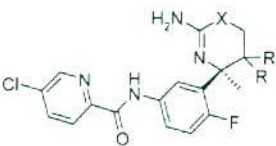
^aReagents and conditions: (a) 36→39: BrCH₂COOEt, Zn, CuCl, THF. 36→90: BrCH₂COOEt, Zn, CuCl, THF. (b) DIBAL, toluene, -76 °C. (c) Trimethyl(trifluoromethyl)silane, THF, TBAF, 0 °C. (d) aq HCl, THF, 22 °C. (e) BrCN, EtOH, 85 °C. (f) H₂SO₄, HNO₃, 0 °C. (g) H₂, Pd/C, EtOH, Et₃N, 22 °C. (h) DMTMM, MeOH, 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-fluoro-benzene (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 Aβ40 reduction in mice at 30 mg/kg ^d (%)
				hBACE1 enzyme	hBACE2 enzyme	Aβ40 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.

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, IC₅₀ = 0.052 μM) 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 Aβ40 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 Aβ40 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 **112**²³ and its fluorine congener **113**,³² both described by Shionogi, were also prepared. Interestingly, thiazine **112** was very potent in vivo (92% Aβ40 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 = ≥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 IC₅₀s values below 0.3 μM, most of them with good cellular activity. The nitrile **14d** displayed already an improved in vivo activity compared to the chloride **14a** with Aβ40 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, V_{ss} = 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 Aβ40 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 (IC₅₀ = 0.027–0.006 μM), 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 Aβ40 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 (IC₅₀ = 0.446/0.110 μM) 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 (IC₅₀ = 0.151, 0.036, and 0.019 μM, respectively). Disappointingly, however, the in vivo activity of **14v** remained rather modest, with a 57% reduction of Aβ40 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

compd	IC ₅₀ (μM) ^a			IC ₅₀ (μM) ^a		shift BACE1 enzyme vs cell-based	brain Aβ40 reduction in mice at 30/10 mg/kg ^c (%)
	hBACE1 enzyme	hBACE2 enzyme	selectivity BACE 2/1	Aβ40 cell-based ^b			
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

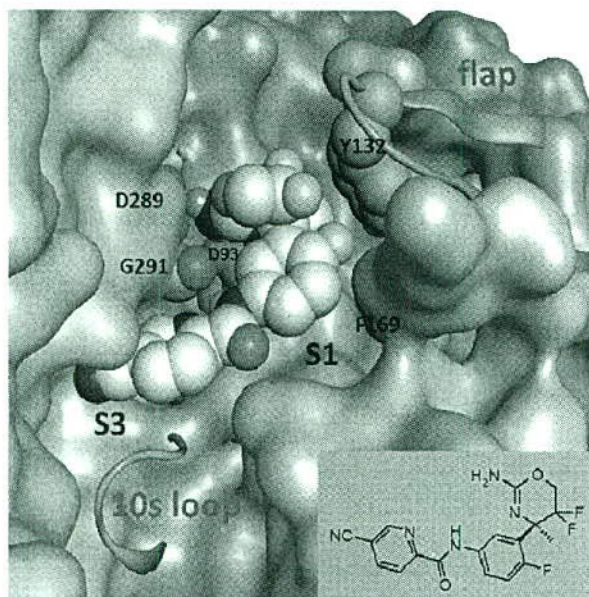


Figure 2. 14d bound to the BACE1 binding site. The amidine recognition motif is tightly bound to the catalytic aspartates D289 and D93 (partially hidden) in the bottom of the pocket. The fluorine atoms in the headgroup and the *o*-F substituent of the phenyl ring (marked in green) form a network of vdW 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.

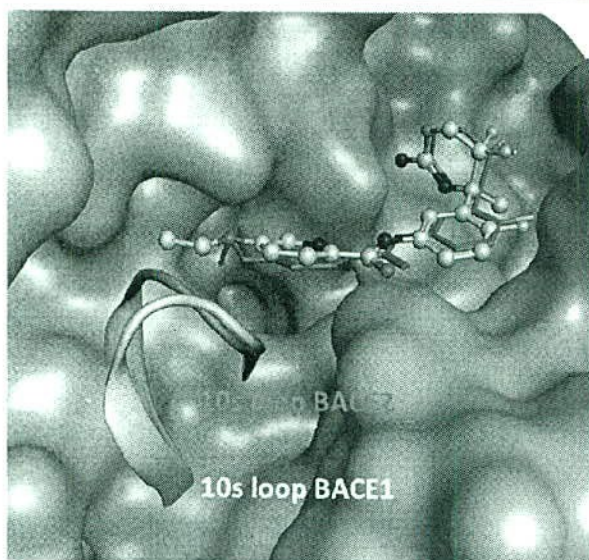


Figure 3. 14i bound to BACE1 (yellow, ball and stick representation) and BACE2 (magenta, stick representation). The molecular surface has been generated for BACE1, and the 10s loops of BACE1 and BACE2 are depicted separately. The ethoxy side chain in 14i prefers a planar orientation around the C–O bond. 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

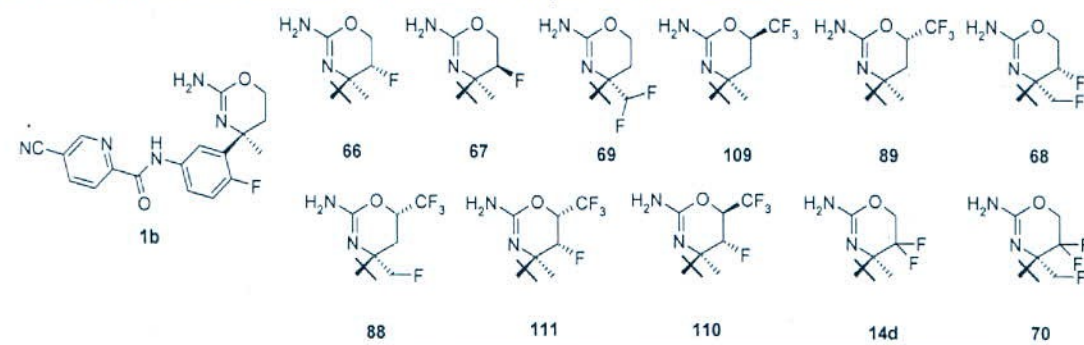
the protonated amidine motif in the oxazine headgroup and the catalytic aspartates. The fluorine atoms in position 5 and the *o*-F substituent of the phenyl ring (S1 pocket) are engaged in a network of van der Waals interactions to Y132 located in the flap and to F169 at the rim of the S1 pocket. A hydrogen 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 IC₅₀ 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,^{47,51} 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 CF₃ 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



compd	pK_a^b	ΔpK_a to 1b	$\log D^c$	$\log P$	$\Delta \log D/\log P$	IC_{50} (μM) ^a		shift BACE1 enzyme vs cell- based	P-gp ER ^e	hERG inhibition at 10 μM (%)	brain A β 40 reduction in mice at 10/3/1 mg/kg ^f (%)
						hBACE1 enzyme	A β 40 cell-based ^d				
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	

^a IC_{50} values are means of at least two independent experiments. ^b pK_a determined by capillary electrophoresis. ^c $\log D$ determined in 1-octanol/phosphate buffer, pH = 7.4. ^dHEK293 cells transfected with wild-type human APP. ^eER = efflux ratio in LLC-PK1 cells stably expressing human MDR1. ^fCompound given orally, $n = 3-4$, 4 h post dose, mean reduction as compared to vehicle control.

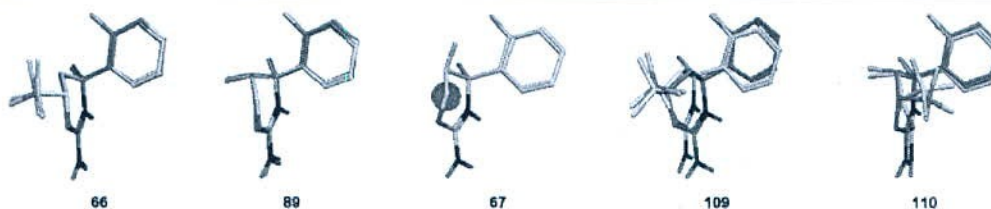


Figure 4. X-ray structures (yellow) of fluorinated head groups with the S1 phenyl ring were energy minimized in the unbound state by a semiempirical geometry optimization with the PM3 Hamiltonian (magenta). The coordinates of 66 and 89 remain almost unchanged as compared to the bound conformation. Therefore, the oxazine rings are without conformational strain. The geometry optimization of unbound 109 and 110 resulted in different ring puckers, indicating that those ligands are not in their lowest energy conformations. For 67, the position of the marked C-atom cannot be clearly assigned because of weak electron density. This indicates the presence of multiple ring conformations.

their impact on the nitrogens. This observation can be rationalized based on the equidistance of the fluorines to both nitrogens in the δ -position having an impact not just on one but on both nitrogens of the amidine moiety. Fluorines located at more than one position (as in 68, 88, 111, 110, and 70⁵¹) showed a more complex picture, with pK_a decrements not being additive.

The lipophilicity of compounds having 1–3 fluorines attached to the headgroup is reduced or similar ($\log P = 1.6-2.5$ in 66–70 and 14d) compared to the nonfluorinated oxazine headgroup 1b ($\log P = 2.3$). Compounds substituted by a CF_3 group showed increased lipophilicity ($\log P = 2.9-3.1$ in 88, 89, 109–111) compared to 1b. As expected, the $\Delta \log P/D$ approaches a value of 0 at a pK_a of 6.3.

The enzymatic potency was strongly affected by fluorines introduced into the oxazine headgroup. A down configured CF_3

group in 89 improved the binding affinity considerably ($IC_{50} = 0.012 \mu M$) compared to unsubstituted 1b ($IC_{50} = 0.051 \mu M$). According to the X-ray structure, the CF_3 group does not interact with BACE1 but points toward the solvent. A possible rationale for this unexpected increase in binding affinity might be the pK_a of 89, which is 2.7 log units lower than for 1b. Therefore, 1b should be more strongly solvated than 89 due to its higher basicity. The lower binding affinity of 1b compared to 89 could be rationalized by the higher desolvation enthalpy upon binding to the active site. The configurations of the fluorine in 66 and the CF_3 group in 89 strongly affected the binding affinity, which increased 16-fold and 65-fold compared to their epimers 67 and 109, respectively. This effect can be rationalized by conformational strain of the head groups. The head groups of the X-ray structures were submitted to a semiempirical geometry optimization⁵² (PM3 Hamiltonian)

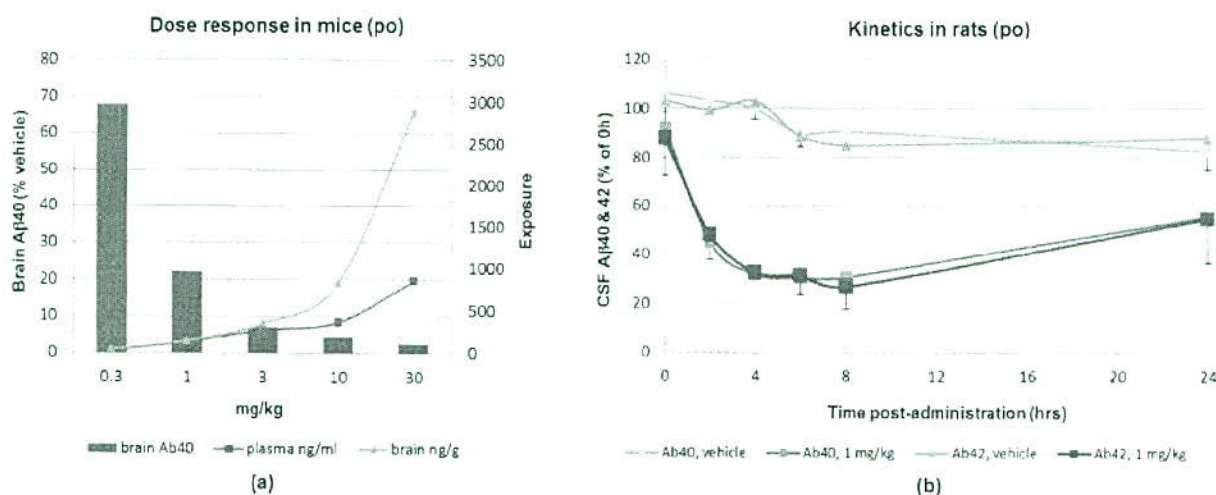


Figure 5. (a) Dose dependent inhibition of brain Aβ40 production in mice by compound 89 determined 4 h oral post dose, $n = 3-4$, mean reduction as compared to vehicle control. (b) Time dependent inhibition of CSF Aβ40/42 production in rats after an oral dose of 1 mg/kg of compound 89, $n = 2-3$. CSF 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: micro-suspension 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-butylsulfinamide (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. (C₁₂H₁₅FN₂O₃S) 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): $m/z = 411.2$ [$\text{M} + \text{H}$] $^+$. Anal. ($\text{C}_{16}\text{H}_{21}\text{F}_3\text{N}_2\text{O}_5\text{S}$) 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-sulfonic 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. ($\text{C}_{14}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_4\text{S}$) 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-sulfonic 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. ($\text{C}_{10}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_4$) 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. ($C_5H_6F_3NO$) 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 (Chiralpack 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. ($C_{12}H_{12}F_4N_2O$) 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. ($C_{12}H_{13}F_4N_3O$) 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.¹⁶ 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 WIPHPFHLVIHTC-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 3× 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, 1× 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 1× 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, 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% Solutol, 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 100000g. 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, f.c.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, pK_a determinations, stability in mouse microsomes, inhibition of CYP_{450w} 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 4J0P (1b), 3ZMG (14d), 4J0T (14i), 4J0V (66), 4J0Y (67), 4J0Z (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

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

HERG, human ether-à-go-go related gene; DCM, dichloromethane; Deoxo-Fluor, bis(2-methoxyethyl)aminosulfur tri-fluoride; DIBAH, 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

■ REFERENCES

- (1) Jakob-Roetne, R.; Jacobsen, H. Alzheimer's Disease: From Pathology to Therapeutic Approaches. *Angew. Chem., Int. Ed.* **2009**, *48*, 3030–3059.
- (2) Hardy, J. A.; Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science* **1992**, *256*, 184–185.
- (3) Selkoe, D. J. Cell biology of the amyloid β -protein precursor and the mechanism of Alzheimer's disease. *Annu. Rev. Cell Biol.* **1994**, *10*, 373–403.
- (4) Selkoe, D. J. The molecular pathology of Alzheimer's disease. *Neuron* **1991**, *6*, 487–498.
- (5) Selkoe, D. J. Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid β -protein. *Ann. N. Y. Acad. Sci.* **2000**, *924*, 17–25.
- (6) Citron, M. Alzheimer's disease: strategies for disease modification. *Nature Rev. Drug Discovery* **2010**, *9*, 387–398.
- (7) Vassar, R. The β -secretase, BACE. A prime drug target for Alzheimer's disease. *J. Mol. Neurosci.* **2001**, *17*, 157–170.
- (8) Cole, S. L.; Vassar, R. BACE1 structure and function in health and Alzheimer's disease. *Curr. Alzheimer Res.* **2008**, *5*, 100–120.
- (9) Cai, H.; Wang, Y.; McCarthy, D.; Wen, H.; Borchelt, D. R.; Price, D. L.; Wong, P. C. BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. *Nature Neurosci.* **2001**, *4*, 233–234.
- (10) Luo, Y.; Bolon, B.; Kahn, S.; Bennett, B. D.; Babu-Khan, S.; Denis, P.; Fan, W.; Kha, H.; Zhang, J.; Gong, Y.; Martin, L.; Louis, J.-C.; Yan, Q.; Richards, W. G.; Citron, M.; Vassar, R. Mice deficient in BACE1, the Alzheimer's β -secretase, have normal phenotype and abolished β -amyloid generation. *Nature Neurosci.* **2001**, *4*, 231–232.
- (11) McConlogue, L.; Buttini, M.; Anderson, J. P.; Brigham, E. F.; Chen, K. S.; Freedman, S. B.; Games, D.; Johnson-Wood, K.; Lee, M.; Zeller, M.; Liu, W.; Motter, R.; Sinha, S. Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP transgenic mice. *J. Biol. Chem.* **2007**, *282*, 26326–26334.
- (12) Rabe, S.; Reichwald, J.; Ammaturo, D.; de, S. B.; Saftig, P.; Neumann, U.; Staufenbiel, M. The Swedish APP mutation alters the effect of genetically reduced BACE1 expression on the APP processing. *J. Neurochem.* **2011**, *119*, 231–239.
- (13) Hu, X.; Hicks, C. W.; He, W.; Wong, P.; Macklin, W. B.; Trapp, B. D.; Yan, R. Bace1 modulates myelination in the central and peripheral nervous system. *Nature Neurosci.* **2006**, *9*, 1520–1525.
- (14) Willem, M.; Garratt, A. N.; Novak, B.; Citron, M.; Kaufmann, S.; Rittger, A.; DeStrooper, B.; Saftig, P.; Birchmeier, C.; Haass, C. Control of Peripheral Nerve Myelination by the β -Secretase BACE1. *Science* **2006**, *314*, 664–666.
- (15) Esterhazy, D.; Stuetzer, I.; Wang, H.; Rechsteiner, M. P.; Beauchamp, J.; Doebeli, H.; Hilpert, H.; Matile, H.; Prummer, M.; Schmidt, A.; Lieske, N.; Boehm, B.; Marselli, L.; Bosco, D.; Kerr-Conte, J.; Aebersold, R.; Spinaz, G. A.; Moch, H.; Migliorini, C.; Stoffel, M. Bace2 Is a β Cell-Enriched Protease that Regulates Pancreatic β Cell Function and Mass. *Cell Metab.* **2011**, *14*, 365–377.
- (16) Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensari, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. Purification and cloning of amyloid precursor protein β -secretase from human brain. *Nature* **1999**, *402*, 537–540.
- (17) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J.-C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. β -Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **1999**, *286*, 735–741.
- (18) Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashler, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Heinrikson, R. L.

- Gurney, M. E. Membrane-anchored aspartyl protease with Alzheimer's disease β -secretase activity. *Nature* **1999**, *402*, 533–537.
- (19) Lin, X.; Koelsch, G.; Wu, S.; Downs, D.; Dashti, A.; Tang, J. Human aspartyl protease memapsin 2 cleaves the β -secretase site of β -amyloid precursor protein. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 1456–1460.
- (20) Hussain, I.; Powell, D.; Howlett, D. R.; Tew, D. G.; Meek, T. D.; Chapman, C.; Gloger, I. S.; Murphy, K. E.; Southan, C. D.; Ryan, D. M.; Smith, T. S.; Simmons, D. L.; Walsh, F. S.; Dingwall, C.; Christie, G. Identification of a Novel Aspartic Protease (Asp 2) as β -Secretase. *Mol. Cell. Neurosci.* **1999**, *14*, 419–427.
- (21) Probst, G.; Xu, Y.-z. Small-molecule BACE1 inhibitors: a patent literature review (2006–2011). *Expert Opin. Ther. Pat.* **2012**, *22*, S11–S40.
- (22) Cohen, N.; Banner, B. L. Synthesis of 2-amino-5,6-dihydro-4H-1,3-thiazines and related compounds by acid catalyzed cyclization of allylic isothiuronium salts. *J. Heterocycl. Chem.* **1977**, *14*, 717–723.
- (23) Kobayashi, N.; Ueda, K.; Itoh, N.; Suzuki, S.; Sakaguchi, G.; Kato, A.; Yukimasa, A.; Hori, A.; Koriyama, Y.; Haraguchi, H.; Yasui, K.; Kanda, Y. Preparation of 2-aminodihydrothiazine derivatives as β -secretase inhibitors. WO2007049532A1, 2007.
- (24) Suzuki, Y.; Motoki, T.; Kaneko, T.; Takaishi, M.; Ishida, T.; Takeda, K.; Kita, Y.; Yamamoto, N.; Khan, A.; Dimopoulos, P. Preparation of condensed aminodihydrothiazine derivatives as inhibitors of β -site APP-cleaving enzyme 1 (BACE1). WO2009091016A1, 2009.
- (25) Audia, J. E.; Mergott, D. J.; Sheehan, S. M.; Watson, B. M. Preparation of aminodihydrothiazine derivatives for use as BACE inhibitors. WO2009134617A1, 2009.
- (26) Chen, J. J.; Zhong, W.; Yang, B.; Qian, W.; Lopez, P.; White, R.; Weiss, M.; Judd, T.; Powers, T.; Cheng, Y.; Dineen, T.; Epstein, O.; Low, J.; Marx, I.; Minatti, A. E.; Paras, N. A.; Potashman, M. Aminodihydrooxazine and aminodihydrothiazine spiro compounds as beta-secretase modulators and their preparation and medical use. WO2011115928A1, 2011.
- (27) Clark, C. T.; Cook, A.; Gunawardana, I. W.; Hunt, K. W.; Kallan, N. C.; Siu, M.; Thomas, A. A.; Volgraf, M. Spirotetrahydronaphthalene derivatives as β -secretase inhibitors and their preparation and use for the treatment of neurodegenerative diseases. WO2011123674A1, 2011.
- (28) May, P. C.; Dean, R. A.; Lowe, S. L.; Martenyi, F.; Sheehan, S. M.; Boggs, L. N.; Monk, S. A.; Mathes, B. M.; Mergott, D. J.; Watson, B. M.; Stout, S. L.; Timm, D. E.; LaBell, E. S.; Gonzales, C. R.; Nakano, M.; Jhee, S. S.; Yen, M.; Ereshefsky, L.; Lindstrom, T. D.; Calligaro, D. O.; Cocke, P. J.; Hall, D. G.; Friedrich, S.; Citron, M.; Audia, J. E. Robust central reduction of amyloid- β in humans with an orally available, non-peptidic β -secretase inhibitor. *J. Neurosci.* **2011**, *31*, 16507–16516.
- (29) Woltering, T. J.; Wostl, W.; Hilpert, H.; Rogers-Evans, M.; Pinard, E.; Mayweg, A.; Banner, D. W.; Benz, J.; Narquizian, R.; Travagli, M.; Power, E.; Pollastrini, M.; Marconi, G.; Gabellieri, E.; Guba, W.; Mauser, H.; Andreini, M.; Jacobsen, H. BACE1 Inhibitors: A Head Group Scan on a Series of Amides. Submitted to *Bioorg. Med. Chem. Lett.*
- (30) Tang, T. P.; Ellman, J. A. The *tert*-Butanesulfinyl Group: An Ideal Chiral Directing Group and Boc-Surrogate for the Asymmetric Synthesis and Applications of β -Amino Acids. *J. Org. Chem.* **1999**, *64*, 12–13.
- (31) Sorochinsky, A.; Voloshin, N.; Markovsky, A.; Belik, M.; Yasuda, N.; Uekusa, H.; Ono, T.; Berbasov, D. O.; Soloshonok, V. A. Convenient Asymmetric Synthesis of β -Substituted α,α -Difluoro- β -amino Acids via Reformatskii Reaction between Davis' *N*-Sulfinylamines and Ethyl Bromodifluoroacetate. *J. Org. Chem.* **2003**, *68*, 7448–7454.
- (32) Tamura, Y.; Suzuki, S.; Tada, Y.; Yonezawa, S.; Fujikoshi, C.; Matsumoto, S.; Koriyama, Y.; Ueno, T. Preparation of aminodihydrothiazine derivatives as BACE1 inhibitors. WO2008133274A1, 2008.
- (33) Masui, M.; Anan, K. Aminothiazine or aminooxazine derivative having amino linker for treatment of Alzheimer disease. WO2011058763A1, 2011.
- (34) Kunishima, M.; Morita, J.; Kawachi, C.; Iwasaki, F.; Terao, K.; Tani, S. Esterification of carboxylic acids with alcohols by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). *Synlett* **1999**, 1255–1256.
- (35) Jaeger, V.; Muller, L.; Schohe, R.; Frey, M.; Ehrler, R.; Hafele, B.; Schroter, D. Syntheses via isoxazolines. Part 18. Old concepts and recent results on stereoselection at isoxazoline ring and adjacent centers. *Lect. Heterocycl. Chem.* **1985**, *8*, 79–98.
- (36) Banner, D.; Hilpert, H.; Mauser, H.; Mayweg, A. V.; Rogers-Evans, M. Preparation of oxazine derivatives as BACE2 inhibitors for treatment of diabetes. WO2011070029A1, 2011.
- (37) Masui, M.; Hori, A. Preparation of 2-amino-4-(aryl or heterocyclyl)-1,3-oxazine derivatives as β -secretase (BACE1) inhibitors. WO2011071135A1, 2011.
- (38) Ellard, J. M.; Farthing, C. N.; Hall, A. Preparation of fused aminodihydrooxazine derivatives as β -amyloid inhibitors and useful in the treatment of neurodegenerative disease caused by $A\beta$ in particular Alzheimer-type dementia. WO2011009898A1, 2011.
- (39) Price, D. A.; Blagg, J.; Jones, L.; Greene, N.; Wager, T. Physicochemical drug properties associated with in vivo toxicological outcomes: a review. *Expert Opin. Drug Metab. Toxicol.* **2009**, *5*, 921–931.
- (40) Peters, J.-U.; Schnider, P.; Mattei, P.; Kansy, M. Pharmacological Promiscuity: Dependence on Compound Properties and Target Specificity in a Set of Recent Roche Compounds. *ChemMedChem* **2009**, *4*, 680–686.
- (41) Peters, J.-U.; Hert, J.; Bissantz, C.; Hillebrecht, A.; Gerebtzoff, G.; Bendels, S.; Tillier, F.; Migeon, J.; Fischer, H.; Guba, W.; Kansy, M. Can we discover pharmacological promiscuity early in the drug discovery process? *Drug Discovery Today* **2012**, *17*, 325–335.
- (42) Mueller, K.; Faeh, C.; Diederich, F. Fluorine in Pharmaceuticals: Looking Beyond Intuition. *Science* **2007**, *317*, 1881–1886.
- (43) Banner, D.; Guba, W.; Hilpert, H.; Mauser, H.; Mayweg, A. V.; Narquizian, R.; Pinard, E.; Power, E.; Rogers-Evans, M.; Woltering, T.; Wostl, W. Preparation of 2-amino-5,5-difluoro-5,6-dihydro-4H-oxazine derivatives as BACE1 and/or BACE2 inhibitors. WO2011069934A1, 2011.
- (44) Patel, S.; Vuillard, L.; Cleasby, A.; Murray, C. W.; Yon, J. Apo and Inhibitor Complex Structures of BACE (β -secretase). *J. Mol. Biol.* **2004**, *343*, 407–416.
- (45) Stauffer, S. R.; Stanton, M. G.; Grego, A. R.; Steinbeiser, M. A.; Shaffer, J. R.; Nantermet, P. G.; Barrow, J. C.; Rittle, K. E.; Collusi, D.; Espeseth, A. S.; Lai, M.-T.; Pietrak, B. L.; Holloway, M. K.; McGaughey, G. B.; Munshi, S. K.; Hochman, J. H.; Simon, A. J.; Selnick, H. G.; Graham, S. L.; Vacca, J. P. Discovery and SAR of isonicotinamide BACE-1 inhibitors that bind β -secretase in a N-terminal 10s-loop down conformation. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1788–1792.
- (46) Ostermann, N.; Eder, J.; Eidhoff, U.; Zink, F.; Hassiepen, U.; Worpenberg, S.; Maibaum, J.; Simic, O.; Hommel, U.; Gerhartz, B. Crystal Structure of Human BACE2 in Complex with a Hydroxyethylamine Transition-State Inhibitor. *J. Mol. Biol.* **2006**, *355*, 249–261.
- (47) Xu, Y.; Li, M.-j.; Greenblatt, H.; Chen, W.; Paz, A.; Dym, O.; Peleg, Y.; Chen, T.; Shen, X.; He, J.; Jiang, H.; Silman, I.; Sussman, J. L. Flexibility of the flap in the active site of BACE1 as revealed by crystal structures and molecular dynamics simulations. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2012**, *D68*, 13–25.
- (48) Banner, D. W.; Gsell, B.; Benz, J.; Bertschinger, J.; Burger, D.; Brack, S.; Cuppuleri, S.; Debulpaep, M.; Gast, A.; Grabulovski, D.; Hennig, M.; Hilpert, H.; Huber, W.; Kuglstatler, A.; Kusznir, E.; Laeremans, T.; Matile, H.; Miscenic, C.; a, Rufer, A.; Schlatter, D.; Staeyert, J.; Stihle, M.; Thoma, R.; Weber, M.; Ruf, A. Mapping the conformational space accessible to BACE2 using surface mutants and co-crystals with Fab-fragments, Fynomers, and Xaperones. *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, in press.

- (49) Jensen, H. H.; Lyngbye, L.; Jensen, A.; Bols, M. Stereoelectronic substituent effects in polyhydroxylated piperidines and hexahydropyridazines. *Chem.—Eur. J.* **2002**, *8*, 1218–1226.
- (50) Morgenthaler, M.; Schweizer, E.; Hoffmann-Roder, A.; Benini, F.; Martin, R. E.; Jaeschke, G.; Wagner, B.; Fischer, H.; Bendels, S.; Zimmerli, D.; Schneider, J.; Diederich, F.; Kansy, M.; Muller, K. Predicting and tuning physicochemical properties in lead optimization: amine basicities. *ChemMedChem* **2007**, *2*, 1100–1115.
- (51) Woltering, T. Halogen-alkyl-1,3 oxazines as BACE1 and/or BACE2 inhibitors and their preparation. WO2012168164A1, 2012.
- (52) VAMP, version 9.0; Accelrys Inc.: San Diego, CA; <http://www.accelrys.com>.
- (53) Rueeger, H.; Lueoend, R.; Rogel, O.; Rondeau, J.-M.; Mobitz, H.; Machauer, R.; Jacobson, L.; Staufienbiel, M.; Desrayaud, S.; Neumann, U. Discovery of Cyclic Sulfone Hydroxyethylamines as Potent and Selective β -Site APP-Cleaving Enzyme 1 (BACE1) Inhibitors: Structure-Based Design and in Vivo Reduction of Amyloid β -Peptides. *J. Med. Chem.* **2012**, *55*, 3364–3386.
- (54) Stamford, A. W.; Scott, J. D.; Li, S. W.; Babu, S.; Tadesse, D.; Hunter, R.; Wu, Y.; Misiaszek, J.; Cumming, J. N.; Gilbert, E. J.; Huang, C.; McKittrick, B. A.; Hong, L.; Guo, T.; Zhu, Z.; Strickland, C.; Orth, P.; Voigt, J. H.; Kennedy, M. E.; Chen, X.; Kuvelkar, R.; Hodgson, R.; Hyde, L. A.; Cox, K.; Favreau, L.; Parker, E. M.; Greenlee, W. J. Discovery of an Orally Available, Brain Penetrant BACE1 Inhibitor That Affords Robust CNS A β Reduction. *ACS Med. Chem. Lett.* **2012**, *3*, 897–902.
- (55) Truong, A. P.; Toth, G.; Probst, G. D.; Sealy, J. M.; Bowers, S.; Wone, D. W. G.; Dressen, D.; Hom, R. K.; Konradi, A. W.; Sham, H. L.; Wu, J.; Peterson, B. T.; Ruslim, L.; Bova, M. P.; Kholodenko, D.; Motter, R. N.; Bard, F.; Santiago, P.; Ni, H.; Chian, D.; Soriano, F.; Cole, T.; Brigham, E. F.; Wong, K.; Zmolek, W.; Goldbach, E.; Samant, B.; Chen, L.; Zhang, H.; Nakamura, D. F.; Quinn, K. P.; Yednock, T. A.; Sauer, J.-M. Design of an orally efficacious hydroxyethylamine (HEA) BACE-1 inhibitor in a preclinical animal model. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6231–6236.
- (56) Aronov, A. M. Ligand structural aspects of hERG channel blockade. *Curr. Top. Med. Chem.* **2008**, *8*, 1113–1127.
- (57) El, M. M.; Haseldonckx, M.; Mackie, C.; Meert, T.; Mercken, M. Method for the determination of the levels of β -amyloid peptide in the CSF sampled from freely moving rats. *J. Pharmacol. Toxicol. Methods* **2005**, *52*, 229–233.
- (58) Gruninger-Leitch, F.; Schlatter, D.; Kung, E.; Nelbock, P.; Dobeli, H. Substrate and inhibitor profile of BACE (β -secretase) and comparison with other mammalian aspartic proteases. *J. Biol. Chem.* **2002**, *277*, 4687–4693.
- (59) Martin, R. E.; Mohr, P.; Maerki, H. P.; Guba, W.; Kuratli, C.; Gavelle, O.; Binggeli, A.; Bendels, S.; Alvarez-Sanchez, R.; Alker, A.; Polonchuk, L.; Christ, A. D. Benzoxazole piperidines as selective and potent somatostatin receptor subtype 5 antagonists. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6106–6113.
- (60) Kuglstatter, A.; Stahl, M.; Peters, J.-U.; Huber, W.; Stihle, M.; Schlatter, D.; Benz, J.; Ruf, A.; Roth, D.; Enderle, T.; Hennig, M. Tyramine fragment binding to BACE-1. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1304–1307.
- (61) Lanz, T. A.; Schachter, J. B. Demonstration of a common artifact in immunosorbent assays of brain extracts: development of a solid-phase extraction protocol to enable measurement of amyloid- β from wild-type rodent brain. *J. Neurosci. Methods* **2006**, *157*, 71–81.
- (62) Brockhaus, M.; Grunberg, J.; Rohrig, S.; Loetscher, H.; Wittenburg, N.; Baumeister, R.; Jacobsen, H.; Haass, C. Caspase-mediated cleavage is not required for the activity of presenilins in amyloidogenesis and NOTCH signaling. *NeuroReport* **1998**, *9*, 1481–1486.

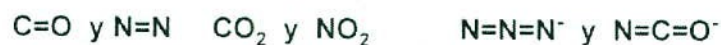
Variaciones basadas en reemplazos bioisostéricos

Isósteros: átomos o grupos de átomos que presentan características electrónicas y estéricas similares

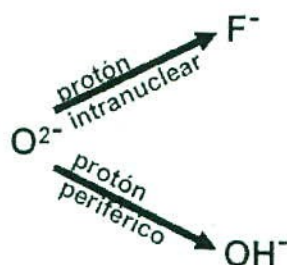
Inicialmente (Langmuir, 1919) se refería a moléculas o átomos con igual número y distribución de electrones:



los que muestran las mayores semejanzas son los isoelectrónicos:



Posteriormente se introdujo el concepto de pseudoátomos que corresponde a agregar un protón a la periferia y no al núcleo:



Erlenmeyer (1932) extendió el concepto de isómero teniendo en cuenta:
todos los elementos presentes en una columna de la tabla periódica
equivalencia de anillos (-CH=CH- equivale a -S-)



similar a



También se amplió el concepto de pseudoátomos

pseudohalógenos: Cl^- , CN^- , SCN^- , etc

Estos constituyen los isómeros clásicos

Criterios actuales de isosterismo

dos moléculas isostéricas deben presentar volúmenes y formas similares

idealmente deberían ser isomórficas y co-cristalizar

los compuestos isostéricos tienen propiedades fisicoquímicas similares pero difieren en ciertas propiedades como momento dipolar, polaridad, polarización.

En química medicinal el concepto de isosterismo se toma en el sentido más amplio

Los isósteros suelen ser más similares en su actividad biológica que en sus propiedades fisicoquímicas

Bioisósteros: (isósteros no clásicos) átomos o grupos de átomos que presentan propiedades biológicas similares (aunque la semejanza fisicoquímica sea vaga)

Isósteros clásicos

1. Átomos y grupos univalentes

- a. CH_3 NH_2 OH F Cl
- b. Cl PH_2 SH
- c. Br $i\text{-Pr}$
- d. I $t\text{-Bu}$

2. Átomos y grupos bivalentes

- a. $-\text{CH}_2-$ $-\text{NH}-$ $-\text{O}-$ $-\text{S}-$ $-\text{Se}-$
- b. $-\text{COCH}_2\text{R}$ $-\text{CONHR}$ $-\text{CO}_2\text{R}$ $-\text{COSR}$

3. Átomos y grupos trivalentes

- a. $-\text{CH}=\text{}$ $-\text{N}=\text{}$
- b. $-\text{P}=\text{}$ $-\text{As}=\text{}$

4. Átomos tetraivalentes

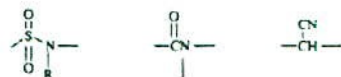
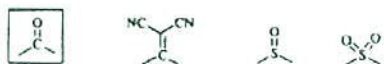
- a. $\begin{array}{c} | \\ -\text{C}- \\ | \end{array}$ $\begin{array}{c} | \\ -\text{Si}- \\ | \end{array}$
- b. $=\text{C}=\text{}$ $\begin{array}{c} + \\ =\text{N}=\end{array}$ $\begin{array}{c} + \\ =\text{P}=\end{array}$

5. Equivalentes de anillos

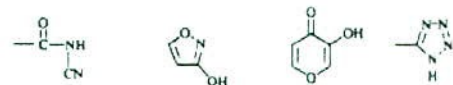
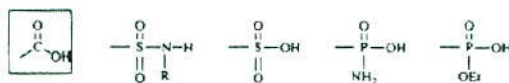
- a. $-\text{CH}=\text{CH}-$ $-\text{S}-$ (ej. benceno, tiofeno)
- b. $-\text{CH}=\text{}$ $-\text{N}=\text{}$ (ej. benceno, piridina)
- c. $-\text{O}-$ $-\text{S}-$ $-\text{CH}_2-$ $-\text{NH}-$ (ej. tetrahidrofurano, tetrahidrotiofeno, ciclopentano, pirrolidina)

Bioisósteros (isósteros no clásicos)

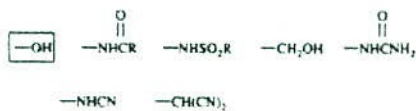
1. Grupo carbonilo



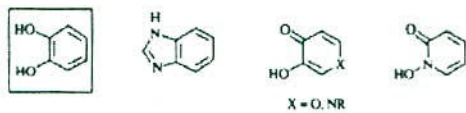
2. Carboxilo



3. Hidroxilo



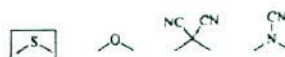
4. Catecol



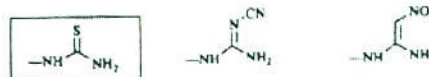
5. Halógeno



6. Tioéter



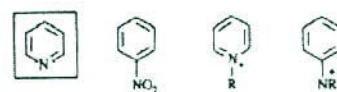
7. Tiourea



8. Azometino



9. Píridina



10. Espaciador



11. Hidrógeno



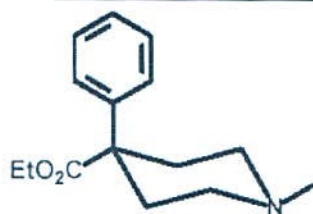
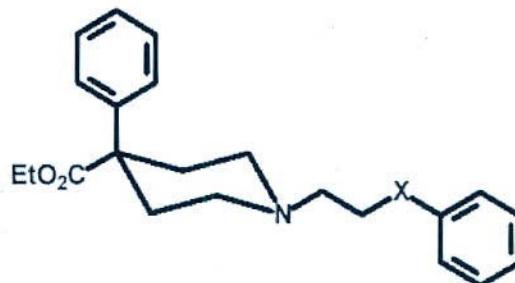
Reemplazo de átomos y grupos univalentes

Los halógenos (especialmente cloro) pueden reemplazarse por otros grupos atractores de electrones como CN y CF₃ (reemplazo muy común)

Intercambio de átomos y grupos divalentes

Corresponde al intercambio en series como O, S, NH y CH₂

X	Act. analgésica (meperidina = 1)
O	12
NH	80
CH ₂	20
S	1,5

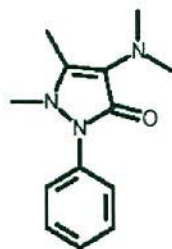


meperidina

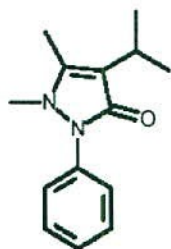
La baja actividad de X = S
puede deberse a la
metabolización más rápida
in vivo (a sulfóxido)

Intercambio de átomos y grupos trivalentes

La sustitución de -CH por -N da muy buenos resultados en cadenas alifáticas

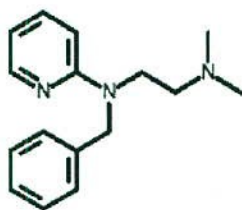


4-dimetilamino-antipirina

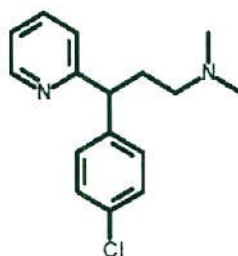


4-isopropilantipirina

derivados de antipirina
con actividad
antipirética similar



tripelenamina

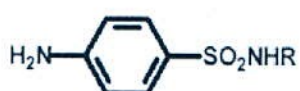


clorfeniramina

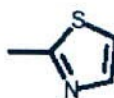
El mismo reemplazo se presenta
al comparar los antihistamínicos
derivados de etilendiamina con las
diarilpropilaminas

Equivalencia entre anillos

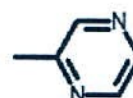
La sustitución de -CH= por -N= y de -CH=CH- por -S- en anillos aromáticos son las aplicaciones más útiles del isoterismo clásico



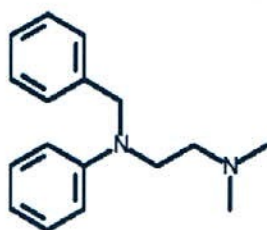
sulfapiridina



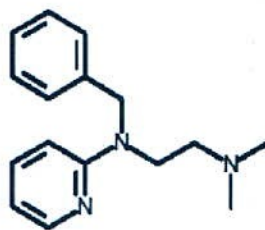
sulfatiazol



sulfapirazina



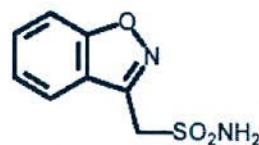
fenbenzamina



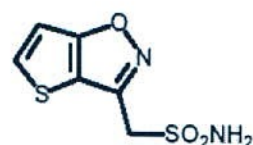
tripelenamina

generalmente no se observan cambios esenciales en la actividad

en ocasiones el reemplazo falla:

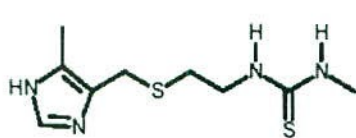


zonisamida
(anticonvulsivo)

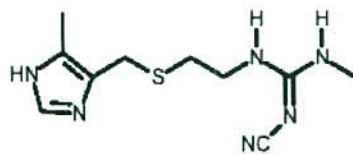


isósterio
(inactivo)

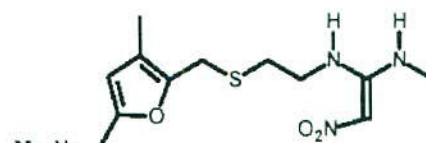
Las copias terapéuticas de cimetidina son un buen ejemplo



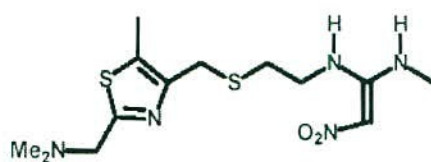
metiamida



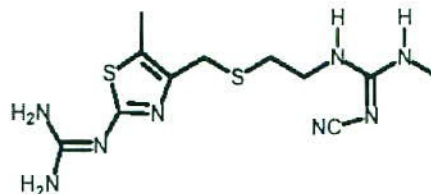
cimetidina



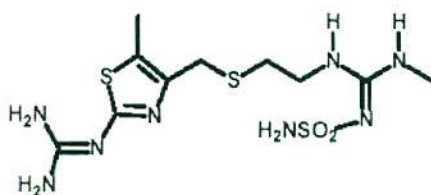
ranitidina



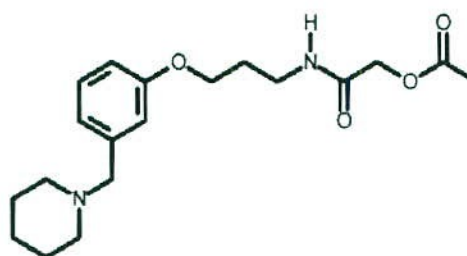
nizatidina



tiotidina

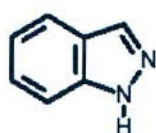
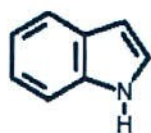


famotidina

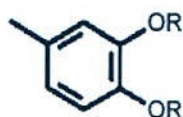
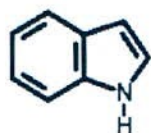


roxatidina

Algunos sistemas de anillos equivalentes

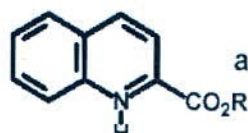
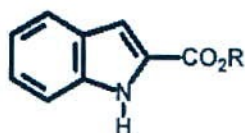


antagonistas de 5-HT₃

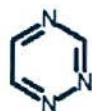


inhibidores de fosfodiesterasa

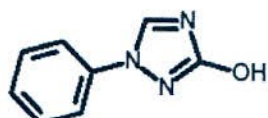
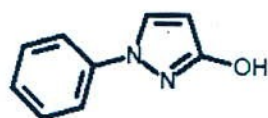
inhibidores de absorción de GABA



antagonistas de glicina



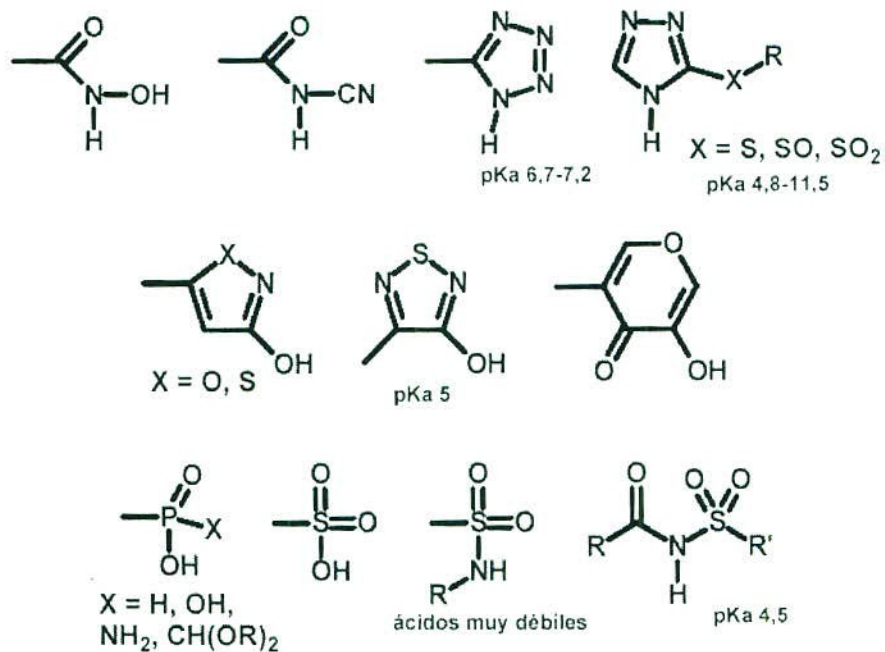
potenciación de 5-HT



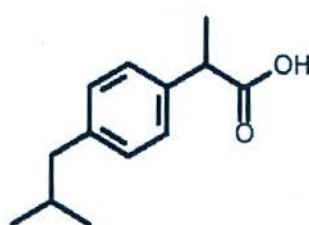
analgésicos

Grupos con efectos polares similares

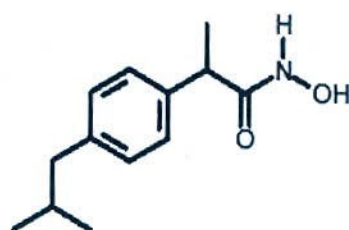
El grupo carboxilo ha sido reemplazado por derivados directos, por heterociclos ácidos planos y por funciones ácidas no planas



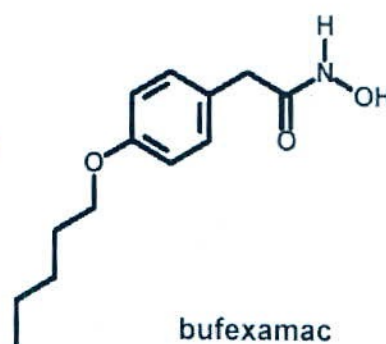
Algunos isómeros de antiinflamatorios



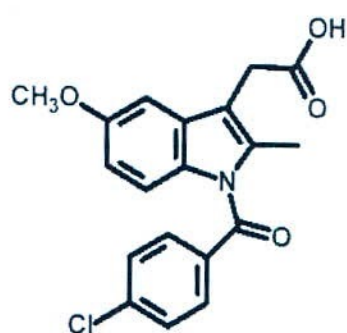
ibuprofeno



ibuproxam
(prodroga de ibuprofeno)



bufexamac

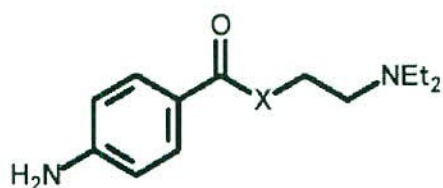


indometacina



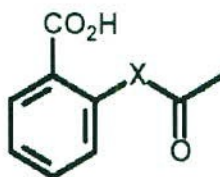
oxametacina

En el isosterismo clásico los ésteres pueden reemplazarse por amidas y las lactonas por lactamas, tiolactonas, lactoles y tiolactoles



X = O procaina (anestésico local)

X = NH procainamida (antiarrítmico, anestésico)



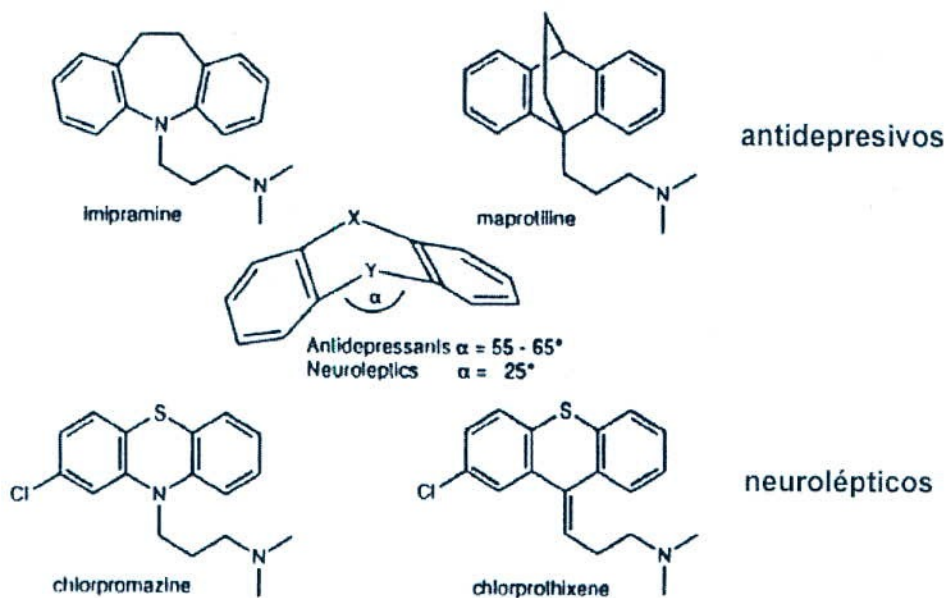
aspirina es un acilante
de la ciclooxigenasa

X = O aspirina (analgésico)

X = S, NH, CH₂ inactivos

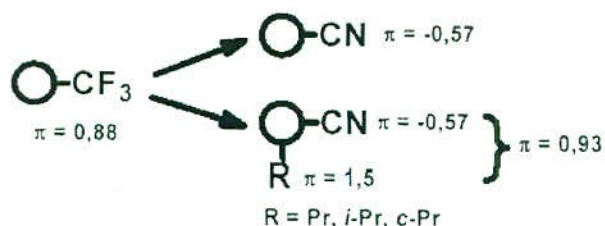
Las modificaciones isostéricas alteran una variedad de parámetros moleculares

Parámetros estructurales: importantes cuando la parte de la molécula involucrada en el cambio isostérico contribuye a una orientación geométrica específica de otros grupos



Parámetros electrónicos: importantes cuando la parte de la molécula involucrada en el cambio isostérico participa en interacciones con el receptor (electrostáticas, transferencia de carga, puente de hidrógeno, etc). Pueden afectar pKa, grado de ionización, etc

Propiedades farmacocinéticas: los parámetros hidrofílicos-lipofílicos, pKa, formación de puentes hidrógeno, etc, son importantes cuando el grupo involucrado en el cambio isostérico participa en la absorción, distribución o excreción de la molécula



El cambio de CF_3 por CN produce una molécula mucho más hidrofílica

Este efecto puede ser compensado agregando un grupo lipofílico en otra parte de la molécula

Propiedades químicas: Son importantes si el grupo reemplazado está involucrado en el bloqueo o ayuda del metabolismo



US008785436B2

(12) **United States Patent**
Hilpert et al.

(10) **Patent No.:** US 8,785,436 B2
(45) **Date of Patent:** Jul. 22, 2014

(54) **1,3-OXAZINES AS BACE 1 AND/OR BACE2 INHIBITORS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 189 days.

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(30) **Foreign Application Priority Data**

May 16, 2011 (EP) 11166208

(51) **Int. Cl.**
C07D 413/04 (2006.01)
C07D 413/10 (2006.01)
C07D 413/12 (2006.01)
A61K 31/5355 (2006.01)

(52) **U.S. Cl.**
USPC 514/228.8; 544/88; 544/96

(58) **Field of Classification Search**
CPC .. C07D 413/04; C07D 413/10; C07D 413/12;
A61K 31/5355
USPC 544/88, 96; 514/228.8
See application file for complete search history.

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WF	2011/071135	6/2011
WO	2007/049532	3/2007
WO	2011/020806	2/2011
WO	2011/058763	5/2011
WO	2011/069934	6/2011
WO	2011/070029	6/2011

OTHER PUBLICATIONS

Zhu et al. *Organic Letters* (2006), 8(12), 2599-2602.*
Basset et al., *Scand. J. Immunol.* 51:307-311 (2000).
Prentki et al., *J. Clin. Invest.* 116(7):1802-1812 (2006).
Kiljanski et al., "Thyroid" 15(7):645-652 (2005).
Luo et al., "Nature Neuroscience" 3:231-232 (2001).
Gatchel et al., *Proc. Natl. Acad. Sci. USA* 105:1291-1296 (2008).

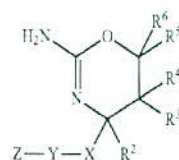
McConlogue et al., "J. Biol. Chem." 282(36):26326-26334 (2007).
Kondoh et al., "Breast Cancer & Research Treatment" 78(1):37-44 (2003).
Kihara et al., *Proc. Natl. Acad. Sci. USA* 106:21807-21812 (2009).
Hussain et al., *Mol. Cell Neurosci.* 16:609-619 (2000).
Talentov et al., "Clin. Cancer Res." 11:7234-7242 (2005).
Hoffmeister et al., "Journal of the Pancreas" 10(5):501-506 (2009).
Kim et al., "Neurobiology of Disease" 22(2):346-356 (2006).
Wild et al., "Diabetes Care" 27(5):1047-1053 (2004).
Finzi et al., "Ultrastruct. Pathol." 32(6):246-251 (2008).
Koistinen et al., "Muscle & Nerve" 34(4):444-450 (2006).
Barbiero et al., *Exp. Neurol.* 182:335-345 (2003).
Baggio et al., *Annu. Rev. Med.* 57:265-281 (2006).
Merten et al., "Zeitschrift für Kardiologie" ((English language Summary is attached to the reference)), 93(11):855-863 (2004).
Hardy et al., *Science* 297:353-356 (2002).
Grewal et al., *Mol. Cell Biol.* 26:4970-4981 (2006).
Vattemi et al., "Lancet" ((9297)), 358:1962-1964 (2001).
Lichtenthaler et al., "J. Biol. Chem." 278(49):48713-48719 (2003).
Maugeri et al., "Srpski Arhiv za Celokupno Lekarstvo" ((Suppl 1)), 138:50-52 (2010).
Lagos et al., "Blood" 109(4):1550-1558 (2007).
Woodard-Grice et al., "J. Biol. Chem." 283(39):26364-26373 (2008).
Kuhn et al., "J. Biol. Chem." 282(16):11982-11995 (2007).
Akpınar et al., *Cell Metab.* 2:385-397 (2005).
Fukui et al., "Cell Metab." 2:373-384 (2005).
Zimmet et al., "Nature" 414:782-282 (2001).
Toegel et al., "Osteoarthritis & Cartilage" 18(2):240-248 (2010).
Hodges et al., *Hum. Mol. Genet.* 15:965-977 (2006).
Li et al., "Aging Cell" 5(2):153-165 (2006).
Vassar et al., *BACE. Science* 286:735 (1999).
Greenberg et al., *Ann. Neurol.* 57:664-678 (2005).
Sugimoto et al., "J. Biol. Chem." 282(48):34896-34903 (2007).
Roberds et al., *Hum. Mol. Genet.* 10(12):1317-1324 (2001).
Hedlund et al., *Cancer Research* 68(2):388-394 (2008).
Desnues et al., *Clin. Vaccine Immunol.* 13:170-178 (2006).
Selkoe et al., "Annual Review Cell Biology" 10:373-403 (1994).
(International Search Report PCT/EP2012/058707 May 11, 2012).

* cited by examiner

Primary Examiner — Kahsay Habte

(57) **ABSTRACT**

The present invention provides compounds of formula I



having BACE1 and/or BACE2 inhibitory activity, their manufacture, pharmaceutical compositions containing them and their use as therapeutically active substances. The active compounds of the present invention are useful in the therapeutic and/or prophylactic treatment of e.g. Alzheimer's disease and type 2 diabetes.

27 Claims, No Drawings

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clopropanecarboxylic acid (3-chloro-quinolin-8-yl)-amide (3.7 mg, 99%) was obtained as a light brown solid. MS (ISP): $m/z=395.0$ $[M+H]^+$.

Example 63

(1R,2R)-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid(3-chloro-quinolin-8-yl)-amide

a) {(R)-4-[(1R,2R)-2-(3-Chloro-quinolin-8-ylcarbamoyl)-cyclopropyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-yl}-carbamic acid tert-butyl ester

In a manner analogous to that described in Example 62.1a), the condensation reaction of (1R,2R)-2-((R)-2-tert-butoxycarbonylamino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropane-carboxylic (intermediate J8.2) with 3-chloro-quinolin-8-ylamine yielded the title compound (36% yield) as a yellow solid. MS (ISP): $m/z=495.2$ $[M+H]^+$.

b) (1R,2R)-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid (3-chloro-quinolin-8-yl)-amide

In a manner analogous to that described in Example 62.1b), the deprotection of {(R)-4-[(1R,2R)-2-(3-chloro-quinolin-8-ylcarbamoyl)-cyclopropyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-yl}-carbamic acid tert-butyl ester with trifluoroacetic acid yielded the title compound (61% yield) as a yellow solid. MS (ISP): $m/z=395.0$ $[M+H]^+$.

Example 64

(1S,2S)-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid(3-chloro-quinolin-8-yl)-amide

a) {(R)-4-[(1S,2S)-2-(3-Chloro-quinolin-8-ylcarbamoyl)-cyclopropyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-yl}-carbamic acid tert-butyl ester

In a manner analogous to that described in Example 62.1a), the condensation reaction of (1S,2S)-2-((R)-2-tert-butoxycarbonylamino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropane-carboxylic (intermediate J8.3) with 3-chloro-quinolin-8-ylamine yielded the title compound (16% yield) as a yellow solid. MS (ISP): $m/z=495.2$ $[M+H]^+$.

b) (1S,2S)-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid (3-chloro-quinolin-8-yl)-amide

In a manner analogous to that described in Example 62.1b), the deprotection of {(R)-4-[(1S,2S)-2-(3-chloro-quinolin-8-ylcarbamoyl)-cyclopropyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-yl}-carbamic acid tert-butyl ester with trifluoroacetic acid yielded the title compound (84% yield) as a yellow solid. MS (ISP): $m/z=395.1$ $[M+H]^+$.

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Example 65

(1R,2R)-2-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid(6-cyano-thieno[3,2-b]pyridin-3-yl)-amide

a) {(R)-4-[(1R,2R)-2-(6-Cyano-thieno[3,2-b]pyridin-3-ylcarbamoyl)-cyclopropyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-yl}-carbamic acid tert-butyl ester

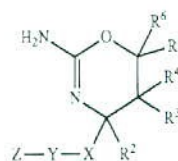
In a manner analogous to that described in Example 62.1a), the condensation reaction of (1R,2R)-2-((R)-2-tert-butoxycarbonylamino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropane-carboxylic (intermediate J8.2) with 3-amino-thieno[3,2-b]pyridine-6-carbonitrile [CAS 116538-96-6; R. Benoit et al. Synthesis 1987(12), 1124-26] yielded the title compound (49% yield) as a yellow solid. MS (ISP): $m/z=492.3$ $[M+H]^+$.

b) (1R,2R)-2-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid(6-cyano-thieno[3,2-b]pyridin-3-yl)-amide

In a manner analogous to that described in Example 62.1b), the deprotection of {(R)-4-[(1R,2R)-2-(6-Cyano-thieno[3,2-b]pyridin-3-ylcarbamoyl)-cyclopropyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-yl}-carbamic acid tert-butyl ester with trifluoroacetic acid yielded the title compound (59% yield) as a light yellow solid. MS (ISP): $m/z=392.2$ $[M+H]^+$.

The invention claimed is:

1. A compound of formula I,



wherein

X is selected from the group consisting of

aryl,
aryl substituted by 1-2 substituents individually selected from R^1 ,
heteroaryl,
heteroaryl substituted by 1-2 substituents individually selected from R^1 and halogen-aryl,
 C_{3-6} -cycloalkyl, and
 C_{3-6} -cycloalkyl substituted by 1-2 substituents individually selected from R^1 ;

Y is selected from the group consisting of

$-C(=O)-NH-$,
 $-CH_2-$,
 $-NH-$,
 $-NH-CH(R^7)-$,
 $-O-CH_2-$, and
absent;

Z is selected from the group consisting of

aryl,
aryl substituted by 1-2 substituents individually selected from R^8 ,

heteroaryl,
 heteroaryl substituted by 1-2 substituents individually
 selected from R⁸,
 C₃₋₆-cycloalkyl,
 C₃₋₆-cycloalkyl substituted by 1-2 substituents individu- 5
 ally selected from R⁸,
 heterocyclyl,
 heterocyclyl substituted by 1-2 substituents individually
 selected from R⁸,
 C₂₋₆-alkynyl, 10
 C₁₋₆-alkyl, and
 C₁₋₆-alkyl substituted by 1-3 substituents individually
 selected from R⁹;
 R¹ is selected from the group consisting of
 hydrogen,
 halogen, and
 C₁₋₆-alkyl;
 R² is selected from the group consisting of
 hydrogen,
 C₁₋₆-alkyl, and
 halogen-C₁₋₃-alkyl;
 R³ is selected from the group consisting of
 hydrogen,
 halogen, and
 C₁₋₆-alkyl;
 R⁴ is selected from the group consisting of
 hydrogen
 halogen, and
 C₁₋₆-alkyl;
 R⁵ is selected from the group consisting of
 hydrogen and
 C₁₋₆-alkyl;
 R⁶ is selected from the group consisting of
 hydrogen and
 C₁₋₆-alkyl;
 R⁷ is selected from the group consisting of
 hydrogen and
 C₁₋₆-alkyl;
 R⁸ is selected from the group consisting of
 halogen,
 cyano,
 C₁₋₆-alkyl,
 halogen-C₁₋₆-alkyl,
 C₁₋₆-alkoxy,
 halogen-C₁₋₆-alkoxy,
 aryl,
 halogen-aryl, and
 C₃₋₆-cycloalkyl; and
 R⁹ is selected from the group consisting of
 halogen,
 cyano,
 C₁₋₆-alkoxy, and
 halogen-C₁₋₆-alkoxy;
 or a pharmaceutically acceptable salt thereof.
 2. The compound according to claim 1
 wherein
 X is selected from the group consisting of
 aryl,
 aryl substituted by 1-2 substituents individually selected 60
 from R¹,
 heteroaryl, and
 heteroaryl substituted by 1-2 substituents individually
 selected from R¹;
 Y is selected from the group consisting of
 —C(=O)—NH—,
 —CH₂—,

—NH—
 —NH—CHR⁷—,
 —O—CH₂—, and
 absent;
 Z is selected from the group consisting of
 aryl,
 aryl substituted by 1-2 substituents individually selected
 from R⁸,
 heteroaryl,
 heteroaryl substituted by 1-2 substituents individually
 selected from R⁸,
 C₃₋₆-cycloalkyl,
 C₃₋₆-cycloalkyl substituted by 1-2 substituents individu-
 ally selected from R⁸,
 heterocyclyl, 15
 heterocyclyl substituted by 1-2 substituents individually
 selected from R⁸, and
 C₂₋₆-alkynyl;
 R¹ is selected from the group consisting of
 hydrogen, 20
 halogen, and
 C₁₋₆-alkyl;
 R² is selected from the group consisting of
 hydrogen,
 C₁₋₆-alkyl, and 25
 halogen-C₁₋₃-alkyl;
 R³ is selected from the group consisting of
 hydrogen,
 halogen, and
 C₁₋₆-alkyl; 30
 R⁴ is selected from the group consisting of
 hydrogen
 halogen, and
 C₁₋₆-alkyl;
 R⁵ is selected from the group consisting of
 hydrogen and 35
 C₁₋₆-alkyl;
 R⁶ is selected from the group consisting of
 hydrogen and
 C₁₋₆-alkyl;
 R⁷ is selected from the group consisting of
 hydrogen and
 C₁₋₆-alkyl; and 40
 R⁸ is selected from the group consisting of
 halogen,
 cyano,
 C₁₋₆-alkyl,
 halogen-C₁₋₆-alkyl,
 C₁₋₆-alkoxy, 45
 halogen-C₁₋₆-alkoxy,
 aryl,
 halogen-aryl, and
 C₃₋₆-cycloalkyl;
 or a pharmaceutically acceptable salt thereof.
 55 3. The compound according to claim 1, wherein R² is
 C₁₋₆-alkyl.
 4. The compound according to claim 3, wherein R² is
 methyl.
 5. The compound according to claim 1, wherein R³ is
 halogen.
 6. The compound according to claim 5, wherein R³ is F.
 7. The compound according to claim 1, wherein R⁴ is H,
 methyl or F.
 8. The compound according to claim 1, wherein R⁵ is H.
 9. The compound according to claim 1, wherein R⁶ is H.
 10. The compound according to claim 1, wherein X is aryl
 substituted by halogen.

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11. The compound according to claim 9, wherein X is phenyl substituted by F.

12. The compound according to claim 1, wherein Y is $\text{—NHCH}_2\text{—}$, —NH— or absent.

13. The compound according to claim 1, wherein Z is heteroaryl substituted by 1-2 substituents individually selected from R^8 and $\text{C}_{3-6}\text{-cycloalkyl}$.

14. The compound according to claims 1, wherein R^8 is selected from the group consisting of

halogen,
cyano,
 $\text{C}_{1-6}\text{-alkyl}$, and
halogen- $\text{C}_{1-6}\text{-alkyl}$.

15. The compound according to claim 1, selected from the group consisting of

(S)-4-{5-[(4-Chloro-1-difluoromethyl-1H-pyrazol-3-yl-methyl)-amino]-2-fluoro-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-5-Fluoro-4-[2-fluoro-5-(2-methoxy-phenylamino)-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-4-[5-(2-Difluoromethoxy-phenylamino)-2-fluoro-phenyl]-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-5-Fluoro-4-[2-fluoro-5-(thiophen-2-ylmethyl)-amino]-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-5-Fluoro-4-[2-fluoro-5-(thiophen-3-ylmethyl)-amino]-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-5-Fluoro-4-[2-fluoro-5-(2-fluoro-benzylamino)-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-4-[5-(Cyclopropylmethyl-amino)-2-fluoro-phenyl]-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-5-Fluoro-4-[2-fluoro-5-[1-(4-fluoro-phenyl)-1H-pyrazol-4-yl]-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-5-Fluoro-4-[2-fluoro-5-(4-methyl-pyrazol-1-yl)-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-4-{5-[(4-Chloro-1-difluoromethyl-1H-pyrazol-3-ylmethyl)-amino]-2-fluoro-phenyl}-5-fluoro-4,5-dimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine, and
(4R,5R)-4-{5-[(4-Chloro-1-methyl-1H-pyrazol-3-ylmethyl)-amino]-2-fluoro-phenyl}-5-fluoro-4,5-dimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
or a pharmaceutical acceptable salt thereof.

16. The compound according to claim 1, selected from the group consisting of

(4R,5R)-5-Fluoro-4-[2-fluoro-5-[1-(2-methyl-5-trifluoromethyl-oxazol-4-yl)-ethylamino]-phenyl]-4,5-dimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(R)-4-[5-[(4-Chloro-1-difluoromethyl-1H-pyrazol-3-yl-methyl)-amino]-2-fluoro-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(R)-4-[5-[1-(4-Chloro-1-methyl-1H-pyrazol-3-yl)-ethylamino]-2-fluoro-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(R)-4-[5-[1-(5-Cyclopropyl-isoxazol-3-yl)-ethylamino]-2-fluoro-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
4-[5-(5-Chloro-pyridin-3-yl)-2-fluoro-phenyl]-4-difluoromethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(R)-4-[5-(5-Chloro-pyridin-3-yl)-2-fluoro-phenyl]-4-difluoromethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,

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(S)-4-[5-(5-Chloro-pyridin-3-yl)-2-fluoro-phenyl]-4-difluoromethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(R)-5,5-Difluoro-4-methyl-4-[3-(3,3,3-trifluoro-propoxy)-phenyl]-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
2-[3-((R)-2-Amino-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenylamino]-benzonitrile,
(R)-5,5-Difluoro-4-(2-fluoro-5-o-tolylamino-phenyl)-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
and
(R)-4-(5-Cyclopentylamino-2-fluoro-phenyl)-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
or a pharmaceutical acceptable salt thereof.

17. The compound according to claim 1, selected from the group consisting of

(R)-4-(3',5'-Dichloro-4-fluoro-biphenyl-3-yl)-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
3'-((R)-2-Amino-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-6-chloro-4'-fluoro-biphenyl-3-carbonitrile,
(R)-4-[5-(1,1-Dioxo-2,3-dihydro-1H-1λ⁶-benzo[b]thiophen-3-ylamino)-2-fluoro-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
5-[3-((R,5R)-2-Amino-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl]-nicotinonitrile,
7-[3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenylamino]-6,7-dihydro-5H-[1]pyridine-3-carbonitrile,
[3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl]-(3-chloro-6,7-dihydro-5H-[1]pyridin-7-yl)-amine,
8-[3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenylamino]-5,6,7,8-tetrahydro-quinoline-3-carbonitrile,
4-[3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-phenoxy-methyl]-benzonitrile,
3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-N-prop-2-ynyl-benzamide,
3-[4-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-pyrazol-1-yl]-benzonitrile, and
(1R,2R)-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid (3-chloro-quinolin-8-yl)-amide,
or a pharmaceutical acceptable salt thereof.

18. The compound according to claim 1, selected from the group consisting of

(1S,2S)-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid (3-chloro-quinolin-8-yl)-amide,
(1R,2R)-2-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid (6-cyano-thieno[3,2-b]pyridin-3-yl)-amide,
(R)-4-(5-[1-(4-Chloro-1-(2,2-difluoro-ethyl)-1H-pyrazol-3-yl)-ethylamino]-2-fluoro-phenyl)-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(4R,5R)-4-[5-(3,5-Difluoro-benzylamino)-2-fluoro-phenyl]-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(R)-4-[3-(5-Chloro-pyridin-2-yl)-7-fluoro-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
(R)-4-[5-(6-Chloro-benzoxazol-2-yl)-2,4-difluoro-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,

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- (R)-5,5-Difluoro-4-{2-fluoro-5-[1-(5-methyl-2H-pyrazol-3-yl)-ethylamino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[1-(2-methyl-5-trifluoromethyl-oxazol-4-yl)-ethylamino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[1-(3-methyl-oxetan-3-yl)-methyl]-amino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-[5-(Cyclopropylmethyl-amino)-2-fluoro-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine, and
 (R)-5,5-Difluoro-4-[2-fluoro-5-(tetrahydro-furan-3-ylamino)-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,

or a pharmaceutical acceptable salt thereof.

19. The compound according to claim 1, selected from the group consisting of

- (R)-5,5-Difluoro-4-[2-fluoro-5-(oxetan-3-ylamino)-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[(tetrahydro-furan-3-yl-methyl)-amino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[1-(4-fluoro-phenyl)-1H-pyrazol-4-yl]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-[5-(5-Chloro-pyridin-3-yl)-2,4-difluoro-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-methyl-4-[3-(4-trifluoromethyl-benzyloxy)-phenyl]-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-methyl-4-[2-(4-methyl-benzyl)-3H-benzimidazol-5-yl]-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-[5-fluoro-2-(4-methoxy-benzyl)-1H-indol-6-yl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-[5-fluoro-2-(4-methyl-benzyl)-3H-indol-6-yl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-[2-fluoro-5-(2-methoxy-phenylamino)-phenyl]-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-[2-fluoro-5-(2-trifluoromethyl-phenylamino)-phenyl]-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-[2-fluoro-5-(6-trifluoromethyl-pyridin-2-ylamino)-phenyl]-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine, and
 (R)-5,5-Difluoro-4-[2-fluoro-5-(3-phenyl-cyclopentylamino)-phenyl]-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,

or a pharmaceutical acceptable salt thereof.

20. The compound according to claim 1, selected from the group consisting of

- (R)-4-[5-(5-Chloro-pyridin-3-yl)-2-fluoro-phenyl]-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-[2-(4-Ethyl-benzyl)-3H-benzimidazol-5-yl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-[5-((R)-2,2-Difluoro-cyclopropylmethoxy)-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,

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- (R)-4-[1-(3-Ethynyl-phenyl)-1H-pyrazol-4-yl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-(2,4-Difluoro-5-pyrimidin-5-yl-phenyl)-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-[5-(2-Chloro-phenylamino)-2-fluoro-phenyl]-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (S)-4-(5-Cyclopentylamino-2-fluoro-phenyl)-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-[3-(4-Chloro-benzyloxy)-phenyl]-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-(3-Cyclopropylmethoxy-phenyl)-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine, and
 (1S,2S)-rel-2-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-cyclopropanecarboxylic acid(3-chloro-quinolin-8-yl)-amide;

or a pharmaceutical acceptable salt thereof.

21. The compound according to claim 1, selected from the group consisting of

- (S)-4-{5-[(4-Chloro-1-difluoromethyl-1H-pyrazol-3-yl-methyl)-amino]-2-fluoro-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-4-[5-(2-Difluoromethoxy-phenylamino)-2-fluoro-phenyl]-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-4-[5-(Cyclopropylmethyl-amino)-2-fluoro-phenyl]-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-4-{5-[(4-Chloro-1-difluoromethyl-1H-pyrazol-3-ylmethyl)-amino]-2-fluoro-phenyl}-5-fluoro-4,5-dimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-4-{5-[(4-Chloro-1-methyl-1H-pyrazol-3-ylmethyl)-amino]-2-fluoro-phenyl}-5-fluoro-4,5-dimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-5-Fluoro-4-[2-fluoro-5-(2-fluoro-benzylamino)-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-5-Fluoro-4-[2-fluoro-5-(2-methoxy-phenylamino)-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-5-Fluoro-4-[2-fluoro-5-(4-methyl-pyrazol-1-yl)-phenyl]-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-5-Fluoro-4-{2-fluoro-5-[(thiophen-2-ylmethyl)-amino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-5-Fluoro-4-{2-fluoro-5-[(thiophen-3-ylmethyl)-amino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine, and
 (4R,5R)-5-Fluoro-4-{2-fluoro-5-[1-(2-methyl-5-trifluoromethyl-oxazol-4-yl)-ethylamino]-phenyl}-4,5-dimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,

or a pharmaceutical acceptable salt thereof.

22. The compound according to claim 1, selected from the group consisting of

- (4R,5R)-5-Fluoro-4-{2-fluoro-5-[1-(4-fluoro-phenyl)-1H-pyrazol-4-yl]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-(2,4-Difluoro-5-pyrimidin-5-yl-phenyl)-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-(3',5'-Dichloro-4-fluoro-biphenyl-3-yl)-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,

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- (R)-4-{5-[1-(4-Chloro-1-(2,2-difluoro-ethyl)-1H-pyrazol-3-yl)-ethylamino]-2-fluoro-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-(5-Cyclopentylamino-2-fluoro-phenyl)-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{3-(4-Chloro-benzyloxy)-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-(5-Chloro-pyridin-2-yl)-7-fluoro-3,4-dihydro-2H-benzof[1,4]oxazin-6-yl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-[1,1-Dioxo-2,3-dihydro-1H-1λ6-benzo[h]thiophen-3-ylamino]-2-fluoro-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-(2-Chloro-phenylamino)-2-fluoro-phenyl}-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-(5-Chloro-pyridin-3-yl)-2,4-difluoro-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine, and
 (R)-4-{5-(5-Chloro-pyridin-3-yl)-2-fluoro-phenyl}-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 or a pharmaceutical acceptable salt thereof.
 23. The compound according to claim 1, selected from the group consisting of
 (R)-4-{5-(6-Chloro-benzooxazol-2-yl)-2,4-difluoro-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-(Cyclopropylmethyl-amino)-2-fluoro-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-[4-(4-Chloro-1-difluoromethyl-1H-pyrazol-3-yl)-methyl]-amino]-2-fluoro-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-[1-(4-Chloro-1-methyl-1H-pyrazol-3-yl)-ethylamino]-2-fluoro-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-4-{5-[1-(5-Cyclopropyl-isoxazol-3-yl)-ethylamino]-2-fluoro-phenyl}-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-o-tolylamino-phenyl}-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-(2-methoxy-phenylamino)-phenyl}-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-(2-trifluoromethyl-phenylamino)-phenyl}-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-(3-phenyl-cyclopentylamino)-phenyl}-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-(6-trifluoromethyl-pyridin-2-ylamino)-phenyl}-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine, and
 (R)-5,5-Difluoro-4-{2-fluoro-5-(oxetan-3-ylamino)-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 or a pharmaceutical acceptable salt thereof.
 24. The compound according to claim 1, selected from the group consisting of
 (R)-5,5-Difluoro-4-{2-fluoro-5-(tetrahydro-furan-3-ylamino)-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{5-fluoro-2-(4-methoxy-benzyl)-1H-indol-6-yl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,

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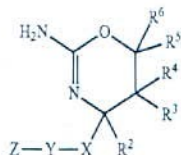
- (R)-5,5-Difluoro-4-{5-fluoro-2-(4-methyl-benzyl)-3H-indol-6-yl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[(3-methyl-oxetan-3-ylmethyl)-amino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[(tetrahydro-furan-3-ylmethyl)-amino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[1-(2-methyl-5-trifluoromethyl-oxazol-4-yl)-ethylamino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[1-(4-fluoro-phenyl)-1H-pyrazol-4-yl]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-{2-fluoro-5-[1-(5-methyl-2H-pyrazol-3-yl)-ethylamino]-phenyl}-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-methyl-4-{2-(4-methyl-benzyl)-3H-benzimidazol-5-yl}-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (R)-5,5-Difluoro-4-methyl-4-{3-(4-trifluoromethyl-benzyloxy)-phenyl}-5,6-dihydro-4H-[1,3]oxazin-2-ylamine, and
 (S)-4-(5-Cyclopentylamino-2-fluoro-phenyl)-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 or a pharmaceutical acceptable salt thereof.
 25. The compound according to claim 1, selected from the group consisting of
 3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl)-(3-chloro-6,7-dihydro-5H-[1]pyridin-7-yl)-amine,
 2-[3-((R)-2-Amino-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenylamino]-benzonitrile,
 3'-((R)-2-Amino-5,5-difluoro-4,6,6-trimethyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-6-chloro-4'-fluoro-biphenyl-3-carbonitrile,
 3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-N-prop-2-ynyl-benzamide,
 4-[3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-phenoxy-methyl]-benzonitrile,
 5-[3-((4R,5R)-2-Amino-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl]-nicotinonitrile,
 7-[3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenylamino]-6,7-dihydro-5H-[1]pyridine-3-carbonitrile, and
 8-[3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenylamino]-5,6,7,8-tetrahydro-quinoline-3-carbonitrile,
 or a pharmaceutical acceptable salt thereof.
 26. The compound according to claim 1, selected from the group consisting of
 (4R,5R)-4-[5-(Cyclopropylmethyl-amino)-2-fluoro-phenyl]-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-4-{5-[(4-Chloro-1-difluoromethyl-1H-pyrazol-3-ylmethyl)-amino]-2-fluoro-phenyl}-5-fluoro-4,5-dimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 (4R,5R)-4-{5-[(4-Chloro-1-methyl-1H-pyrazol-3-ylmethyl)-amino]-2-fluoro-phenyl}-5-fluoro-4,5-dimethyl-5,6-dihydro-4H-[1,3]oxazin-2-ylamine,
 [3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl)-(3-chloro-6,7-dihydro-5H-[1]pyridin-7-yl)-amine,

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5-[3-((4R,5R)-2-Amino-5-fluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl]-nicotinonitrile, and

7-[3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenylamino]-6,7-dihydro-5H-[1]pyridine-3-carbonitrile.

27. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula I



wherein

X is selected from the group consisting of

aryl,

aryl substituted by 1-2 substituents individually selected from R¹,

heteroaryl,

heteroaryl substituted by 1-2 substituents individually selected from R¹ and halogen-aryl,

C₃₋₆-cycloalkyl, and

C₃₋₆-cycloalkyl substituted by 1-2 substituents individually selected from R¹;

Y is selected from the group consisting of

—C(=O)—NH—,

—CH₂—,

—NH—

—NH—CHR⁷—,

—O—CH₂—, and

absent;

Z is selected from the group consisting of

aryl,

aryl substituted by 1-2 substituents individually selected from R⁸,

heteroaryl,

heteroaryl substituted by 1-2 substituents individually selected from R⁸,

C₃₋₆-cycloalkyl,

C₃₋₆-cycloalkyl substituted by 1-2 substituents individually selected from R⁸,

heterocyclyl,

heterocyclyl substituted by 1-2 substituents individually selected from R⁸,

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C₂₋₆-alkynyl,

C₁₋₆-alkyl, and

C₁₋₆-alkyl substituted by 1-3 substituents individually selected from R⁹;

R¹ is selected from the group consisting of hydrogen, halogen, and C₁₋₆-alkyl;

R² is selected from the group consisting of hydrogen, C₁₋₆-alkyl, and halogen-C₁₋₃-alkyl;

R³ is selected from the group consisting of hydrogen, halogen, and C₁₋₆-alkyl;

R⁴ is selected from the group consisting of hydrogen, halogen, and C₁₋₆-alkyl;

R⁵ is selected from the group consisting of hydrogen and C₁₋₆-alkyl;

R⁶ is selected from the group consisting of hydrogen and C₁₋₆-alkyl;

R⁷ is selected from the group consisting of hydrogen and C₁₋₆-alkyl;

R⁸ is selected from the group consisting of halogen, cyano, C₁₋₆-alkyl, halogen-C₁₋₆-alkyl, C₁₋₆-alkoxy, halogen-C₁₋₆-alkoxy, aryl,

halogen-aryl, and C₃₋₆-cycloalkyl; and

R⁹ is selected from the group consisting of halogen, cyano, C₁₋₆-alkoxy, and halogen-C₁₋₆-alkoxy;

or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

* * * * *



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EXAMINER

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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

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)

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13/467,085	05/09/2012	Hans Hilpert	111890-83098 (27315 US)	5486

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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, 1 _____
- (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. 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. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

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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 AllowabilityApplication No.
13/467,085Applicant(s)
HILPERT ET AL.Examiner
KAHSAY HABTEArt Unit
1624AIA (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)
2. ☒ Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date 7/9/12 & 6/29/12
3. ☐ Examiner's Comment Regarding Requirement for Deposit
of Biological Material
4. ☐ Interview Summary (PTO-413),
Paper No./Mail Date _____
5. ☐ Examiner's Amendment/Comment
6. ☒ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____

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