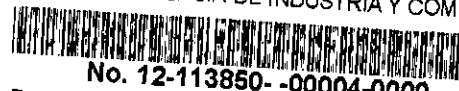


LLOREDA CAMACHO & CO.

ABOGADOS

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 12-113850- -00004-0000

Fecha: 2014-10-15 16:23:38 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 523 RESPUESTA Folios: 27

Trámite: 11
Evento: 378
Actuación: 523

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: PI.-F. HOFFMANN-LA ROCHE AG Y SIENA BIOTECH S.P.A.-Solicitud de
Patente de Invención en Fase Nacional PCT para "2-AMINO-5,5-DIFLUOR-5,6-
DIHIDRO-4H-OXAZINAS COMO INHIBIDORES DE BACE 1 Y/O BACE 2".-
Clase C07D 265/08.-Expediente número 12-113.850.-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 22 de julio de 2014.

2. Con el fin de responder las objeciones expuestas por el Señor Examinador en su Concepto Técnico correspondiente al Oficio No. **8504**, en relación con las excepciones a la patentabilidad, la claridad del capítulo reivindicatorio y el nivel Inventivo de la presente solicitud, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos por medio de los cuales no sólo se superan completamente dichas objeciones sino que además se establece de manera incontrovertible la patentabilidad de la solicitud en estudio.

3. **CAPÍTULO REIVINDICATORIO.**

3.1. **MODIFICACIONES AL CAPÍTULO REIVINDICATORIO.**

Inicialmente, me permito manifestar que es deseo del solicitante presentar un **capítulo reivindicatorio modificado**, que remplace al actualmente presentado, esto con el fin de superar las objeciones presentadas como parte del concepto técnico realizado por ese Despacho.

En conexión con el mencionado **capítulo reivindicatorio modificado** me permito resaltar que este no se constituye de ninguna manera en una ampliación al alcance de la invención, tal como fue originalmente presentada, toda vez que dicho capítulo define los

compuestos que son objeto de la presente invención, los cuales están plenamente soportados en la descripción, tales modificaciones se exponen a continuación:

- **Reivindicaciones 1 a 5.** Corresponden respectivamente a las reivindicaciones 1 a 5 actualmente presentadas, sin embargo se ha limitado la definición de alquilo inferior, alcoxi inferior, alquenilo inferior y alquinilo inferior especificando la cantidad de carbonos, de manera que ahora se hace referencia a alquilo C₁₋₆, alcoxi C₁₋₆, alquenilo C₂₋₆, y alquinilo C₂₋₆.

Adicionalmente, se ha eliminado el término "opcionalmente sustituido" de la reivindicación 4.

- **Reivindicaciones 6 a 18.** Corresponden respectivamente a las reivindicaciones 6 a 18 actualmente presentadas.

- **Nueva Reivindicación 19:** Hace referencia específicamente al compuesto clorhidrato de la [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-ciano-piridina-2-carboxílico. Sustento en el ejemplo 11 del capítulo descriptivo.

Las reivindicaciones **19 a 24** actualmente presentadas se han eliminado del capítulo reivindicatorio.

De esta forma, el **capítulo reivindicatorio modificado** está constituido por **19 reivindicaciones** que se refieren de manera más clara y concisa a las características técnicas esenciales de los compuestos que son objeto de la presente solicitud, de acuerdo con las enseñanzas de la descripción.

3.2. CLARIDAD DEL CAPÍTULO REIVINDICATORIO.

- Ahora bien, el señor Examinador afirma que la reivindicación 4 no es clara porque contiene el término "opcionalmente". En este sentido y tal como se señaló en el numeral anterior, el término objetado ha sido eliminado de la reivindicación 4 que se presenta como parte del capítulo reivindicatorio modificado.

- Asimismo, el señor examinador ha objetado las reivindicaciones 21 y 23, dado que hacen referencia a un uso y a una composición sin definir sus ingredientes y proporciones.

Al respecto, me permito resaltar que las reivindicaciones 21 y 23 han sido eliminadas del capítulo reivindicatorio modificado.

LLOREDA CAMACHO & CO.

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

• Por otro lado, el señor examinador señala que las reivindicaciones 1-7 no son concisas y sugiere restringir la solicitud al tipo de sustituyentes que han sido probados, es decir, que se encuentran debidamente sustentados.

Tomando en cuenta la sugerencia del señor Examinador, en primer lugar me permito señalar que como bien se detalla en el numeral 3.1, el término "inferior" ha sido eliminado, y en su lugar se ha especificado el número de carbonos correspondiente a alquilo, alcoxi, alquenilo y alquinilo.

En segundo lugar, me permito manifestar que los compuestos que se reclaman en el capítulo reivindicatorio modificado se encuentran plenamente sustentados en la descripción, principalmente en la tabla comprendida entre las páginas 61 a 76, y a lo largo de los ejemplos de las páginas 86 a 142.

Por lo tanto solicito amablemente a ese Despacho sea retirada dicha objeción sobre el capítulo reivindicatorio.

En vista de lo anterior, resulta evidente que el capítulo reivindicatorio modificado supera completamente las objeciones presentadas por el Señor Examinador y se enmarca completamente dentro de lo preceptuado en el Artículo 30 de la Decisión Andina 486 de 2000.

4. **EXCEPCIÓN A LA PATENTABILIDAD.**

Por otro lado, el Señor Examinador indica que el objeto de las reivindicaciones 22 a 24 actualmente presentadas no sería patentable dado que se encuentran referidas a un método de tratamiento.

Sobre este particular me permito señalar que las reivindicaciones 22 a 24 se han eliminado, y por lo tanto el capítulo reivindicatorio modificado no está de ninguna manera dirigido a materia no patentable.

Por lo tanto me permito señalar que el mencionado capítulo reivindicatorio modificado se ajusta completamente a los requerimientos del Artículo 20 de la Decisión Andina 486 de 2000.

5. **NIVEL INVENTIVO.**

Finalmente, el Señor Examinador considera que los compuestos reclamados en la presente solicitud carecerían de altura inventiva con respecto a las enseñanzas de los documentos **EP 1942105 (D1)** y **WO 0125229 (D2)**.

A este respecto el Señor Examinador asegura que la persona del oficio normalmente versada en la materia, incluiría como sustituyente del grupo carbamoilo al fenilo de acuerdo con lo divulgado en el documento D2 dentro del grupo carbamoilo del compuesto revelado en el documento D1, para llegar así al objeto de las reivindicaciones 1 y 3 de la solicitud.

Así mismo, señala que la persona del oficio normalmente versada en la materia, incluiría el anillo oxazino de acuerdo con lo divulgado en el documento D1, dentro del proceso de obtención revelado en el documento D2, para llegar así al objeto de la reivindicación 19 de la solicitud.

Respecto a lo anterior, me permito señalar que las enseñanzas de los mencionados documentos D1 y D2, de ninguna manera pueden conducir a las estructuras particulares de los compuestos de la invención, tal y como se reclaman dentro del capítulo reivindicatorio modificado que se presenta junto con este memorial.

En este sentido, en principio cabe señalar que el documento D1 reclama compuestos que comprenden una porción de su estructura que es similar a la de los compuestos de la presente invención, pero permite la sustitución de la misma de varias maneras que conducen a un gran número de compuestos con un patrón de sustitución diferente.

En este sentido, es importante resaltar que el documento D1 da 1282 ejemplos que combinan los diferentes radicales de la molécula de varias maneras. Particularmente, la Tabla 170 en la página 260 de D1, muestra distintos valores de IC_{50} para ejemplos seleccionados, y además en esa misma página del documento D1 menciona que otros compuestos ejemplificados presentan valores de IC_{50} de menos de 100 μM . En este sentido, es claro que el experto en la materia se concentraría en aquellos ejemplos que presentan datos biológicos.

Se tiene entonces que el compuesto con mayor potencia que es divulgado en el documento D1 es el compuesto 1043, el cual presenta un valor de IC_{50} de 0.027 μM , seguido muy de cerca por los compuestos 1199 (IC_{50} : 0.032 μM), 1100 (IC_{50} : 0.037 μM) y 165 (IC_{50} : 0.0394 μM).

En este orden de ideas, cabe destacar que los 4 ejemplos de D1 mencionados anteriormente muestran un patrón de sustitución muy similar. Específicamente los 4 ejemplos **presentan como parte de su estructura un radical tiazinilo**. Igualmente, es claro que el documento **D1 enseña que los derivados de tiazinilo son el grupo preferido de la invención revela en tal documento**. Incluso, el mismo título del documento D1 apoya la hipótesis de que un experto en la materia con interés en el desarrollo de inhibidores de BACE1 adecuados debería concentrarse en los compuestos derivados de tiazinilo amino-sustituidos.

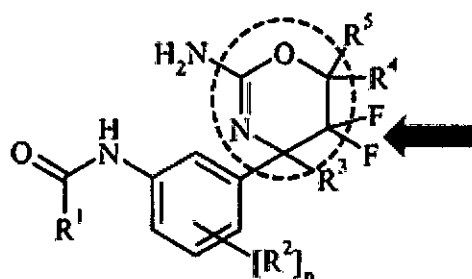
LLOREDA CAMACHO & CO.

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

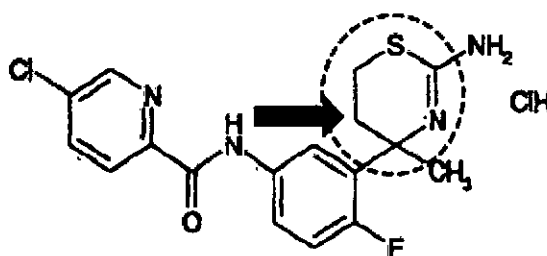
En síntesis, los compuestos reclamados en la presente solicitud difieren de las enseñanzas divulgadas en el documento D1 en dos aspectos importantes:

(i) **los compuestos de la invención presentan un grupo oxazinilo** (ver círculo en rojo), **mientras que los compuestos divulgados en D1 presentan como parte fundamental de su molécula un grupo tiazinilo** (ver círculo en rojo), y

(ii) **el anillo oxazinilo en los compuestos de la invención está disustituídos en la posición 5 por fluor** (5,5-difluor, ver flecha verde), mientras que los compuestos de D1 no presentan tal sustitución.



Compuestos de la presente
invención



Compuesto 1043 de D1

Lo anterior significa que la persona del oficio medianamente versada en la materia tiene que realizar dos tipos diferentes de modificaciones a la molécula de tiazinilo amino-sustituido de D1, para llegar a los compuestos que se reclaman en la presente solicitud. En este sentido, el documento D1 no brinda ninguna sugerencia acerca de que estas modificaciones deben realizarse con el fin de llegar a obtener compuestos más adecuados.

Cabe agregar que si bien, el documento D1 hace referencia a 3 compuestos que poseen un resto oxazinilo (compuestos 654, 783, 878) de un total de 1285, es importante señalar que en tal documento no se encuentra ninguna información acerca de la actividad de esos compuestos sobre BACE1. Por lo tanto, la persona experta en la materia simplemente deduciría que los compuestos de D1 que poseen un resto oxazinilo son menos adecuados que aquellos que presentan el grupo tiazinilo.

Asimismo, **ninguno de los 1285 compuestos ejemplificados en D1 presenta una disustitución con flúor sobre el núcleo central**, es más no existe ninguna enseñanza en D1 que hubiese motivado a la persona experta en la materia enfrentada con el problema técnico objetivo, a que modificara o adaptara D1 para llegar a la presente invención.

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

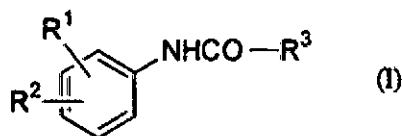
Table 1. In Vitro and in Vivo Profiles of Oxazines 1a and 14a and Thiazines 112 and 113

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

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

Tabla 1, Documento Hilpert et al.

Ahora bien, cabe resaltar que el documento D2 se refiere a compuestos derivados de amida que poseen una estructura química completamente diferente a la de los compuestos aquí reclamados.



Compuestos divulgados en D2

Adicionalmente, es de resaltar que los compuestos que enseña el documento D2 tienen propiedades como antagonistas de 5HT y de ninguna manera son antagonistas BACE1 activos.

En vista de todo lo anterior, es evidente que las enseñanzas del documento D2 de ninguna manera complementan las enseñanzas de D1 para llegar a los compuestos de la invención, mucho más si se tiene en cuenta que los compuestos divulgados en **D2 son antagonistas de 5HT**, es decir, están dirigidos a un objetivo diferente al de los compuestos de la invención, los cuales son potentes **inhibidores de BACE**.

Sin embargo, 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 D1 mediante las enseñanzas divulgadas en D2, sino que si lo hubiera hecho de esa manera, por

LLOREDA CAMACHO & CO.

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

qué el documento D1 le motivó a hacerlo con la expectativa de resolver el problema técnico objetivo?

Dado que no existe tal motivación, es evidente que los compuestos reclamados en la presente solicitud son inventivos frente a la combinación de las enseñanzas de los documentos D1 y D2.

Por último, muy respetuosamente me permito señalar que la única motivación que tendría la persona versada en la materia para llegar a los compuestos aquí reclamados, 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", toda vez que conociendo de antemano la invención el Señor Examinador está seleccionado partes específicas de las enseñanzas contenidas dentro de D1 y D2, y modificándolas de manera que permitan construir los compuestos reclamados por la presente solicitud, pasos que de ninguna manera 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 D1 y D2 parezcan obvias, toda vez que ya se sabe de antemano que efectivamente estas modificaciones resuelven 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.

Finalmente, dentro del contexto de lo discutido a lo largo del presente memorial me permito referirme al señalamiento que hace el Tribunal de Justicia de la Comunidad Andina con respecto a las definiciones de "obvio" y "evidente" en la evaluación del Nivel Inventivo de una solicitud de patente*:

"Resulta pertinente, a los efectos del artículo 4 de la Decisión 344, definir qué se entiende por "obvio" y por "evidente" a fin de determinar qué se debe entender por nivel inventivo.

Lo obvio es aquello que salta a la vista de manera clara y directa; lo "que se encuentra o pone delante de los ojos, muy claro o que no tiene dificultad". Mientras que lo evidente consiste en la "certeza clara y manifiesta de la que no se puede dudar; cierto, claro, patente y sin la menor duda". De forma que algo que resulte obvio no es necesariamente evidente; empero lo que es evidente, es también obvio.

* Tribunal de Justicia de la Comunidad Andina, PROCESO 192-IP-2005

En este orden de ideas, se puede concluir que una invención goza de nivel inventivo cuando a los ojos de un experto medio en el asunto de que se trate, se necesita algo más que la simple aplicación de los conocimientos técnicos en la materia para llegar a ella, es decir, que de conformidad con el estado de la técnica el invento no sea consecuencia clara y directa de dicho estado."

De dicho señalamiento es claro que dentro de la Comunidad Andina, para que un documento afecte el nivel inventivo de una solicitud de patente el objeto de dicha solicitud debe ser derivable de manera evidente a partir de las enseñanzas del arte previo, y dicho documento debe brindar una motivación y guía suficientes que conduzcan inevitablemente a obtener la invención.

En vista de lo anterior, dado que a partir de las enseñanzas de los documentos D1 y D2 no "saltan a la vista de manera clara y directa" los compuestos que son objeto de la presente invención, y al no tenerse "certeza clara y manifiesta" a partir de las enseñanzas de dichos documentos de que dichos compuestos serían inhibidores adecuados de BACE 1 y/o BACE2, es claro que la solicitud en estudio es a todas luces inventiva con respecto al estado del arte más próximo.

6. **CONCLUSIÓN.**

Para finalizar me permito reiterar que el capítulo reivindicatorio modificado presentado junto con este memorial es totalmente claro y conciso al reclamar los compuestos de la invención sin hacer referencia a excepciones a la patentabilidad cumpliendo así con lo exigido por la mencionada Decisión Andina 486 de 2000.

Por otro lado, en virtud de los argumentos discutidos con respecto a los documentos citados, se estableció de manera incontrovertible que los compuestos que son objeto de la solicitud en estudio, de ninguna manera resultarían evidentes para la persona versada en la materia a partir de las enseñanzas de los documentos D1 y D2, demostrando irrefutablemente la patentabilidad de la presente solicitud.

7. Anexo me permito presentar:

- i) Copia de las páginas **143 a 156** que corresponde al **Capítulo reivindicatorio modificado**, tal como se discutió en el presente memorial, constituido por **19** reivindicaciones, y
- ii) 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.

LLOREDA CAMACHO & CO.

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

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

Señor Superintendente,



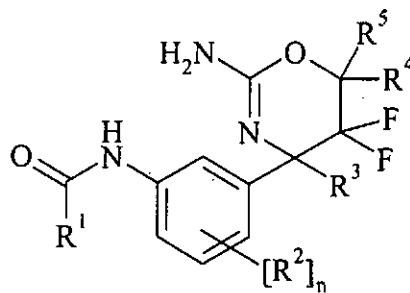
ALICIA LLOREDA RICAURTE

T. P. 53.215

Dirección para Notificaciones: notificacionespatentes@lloredacamacho.com

REIVINDICACIONES

1. Un compuesto de la fórmula I,



en la que

5 R^1 se elige entre el grupo formado por

- i) arilo,
- ii) arilo sustituido por 1-4 sustituyentes elegidos individualmente entre ciano, ciano-alquilo C_{1-6} , halógeno, halógeno-alcoxi C_{1-6} , halógeno-alquilo C_{1-6} , alcoxi C_{1-6} , alcoxi C_{1-6} -alquilo C_{1-6} y alquilo C_{1-6} ,
- 10 iii) heteroarilo y
- iv) heteroarilo sustituido por 1-4 sustituyentes elegidos individualmente entre amido, ciano, ciano-alquilo C_{1-6} , cicloalquilo, cicloalquil-alquenilo C_{2-6} , cicloalquil-alquinilo C_{2-6} , cicloalquil-alquilo C_{1-6} , cicloalquil-alcoxi C_{1-6} , halógeno, halógeno-alcoxi C_{1-6} , halógeno-alquilo C_{1-6} , alcoxi C_{1-6} , alcoxi C_{1-6} -alquilo C_{1-6} , alquenilo C_{2-6} , alquinilo C_{2-6} , alquilo C_{1-6} y nitro;
- 15 v) alquilo C_{1-6} ,
- vi) alquilo C_{1-6} sustituido por 1-5 sustituyentes elegidos individualmente entre ciano, halógeno, hidroxí y alcoxi C_{1-6} ;
- vii) alquenilo C_{2-6} ,
- 20 viii) alquenilo C_{2-6} sustituido por 1-4 sustituyentes elegidos individualmente entre ciano, ciano-alquilo C_{1-6} , halógeno, halógeno-alcoxi C_{1-6} , halógeno-alquilo C_{1-6} , heteroarilo, alcoxi C_{1-6} , alcoxi C_{1-6} -alquilo C_{1-6} y alquilo C_{1-6} ,
- ix) cicloalquilo,
- x) cicloalquilo sustituido por 1-4 sustituyentes elegidos individualmente entre
- 25 ciano, ciano-alquilo C_{1-6} , halógeno, halógeno-alcoxi C_{1-6} , halógeno-alquilo C_{1-6} , alcoxi C_{1-6} , alcoxi C_{1-6} -alquilo C_{1-6} y alquilo C_{1-6} ,

R^2 se elige entre el grupo formado por

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

R³ es alquilo C₁₋₆;

5 R⁴ se elige entre el grupo formado por

- i) hidrógeno y
- ii) alquilo C₁₋₆;

R⁵ se elige entre el grupo formado por

- i) hidrógeno y
- 10 ii) alquilo C₁₋₆;

n es el número 0, 1 ó 2;

o sus sales farmacéuticamente aceptables.

2. Un compuesto de la fórmula I según la reivindicación 1, en la que

R¹ se elige entre el grupo formado por

- 15 i) heteroarilo y
- ii) heteroarilo sustituido por 1-4 sustituyentes elegidos individualmente entre amido, ciano, ciano-alquilo C₁₋₆, cicloalquilo, cicloalquil-alquenilo C₂₋₆, cicloalquil-alquinilo C₂₋₆, cicloalquil-alquilo C₁₋₆, cicloalquil-alcoxi C₁₋₆, halógeno, halógeno-alcoxi C₁₋₆, halógeno-alquilo C₁₋₆, alcoxi C₁₋₆, alcoxi C₁₋₆-alquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆, alquilo C₁₋₆ y nitro;
- 20 iii) alquilo C₁₋₆,
- iv) alquilo C₁₋₆ sustituido por 1-5 sustituyentes elegidos individualmente entre ciano, halógeno, hidroxilo y alcoxi C₁₋₆;
- v) alquenilo C₂₋₆,
- 25 vi) alquenilo C₂₋₆ sustituido por 1-4 sustituyentes elegidos individualmente entre ciano, ciano-alquilo C₁₋₆, halógeno, halógeno-alcoxi C₁₋₆, halógeno-alquilo C₁₋₆, heteroarilo, alcoxi C₁₋₆, alcoxi C₁₋₆-alquilo C₁₋₆ y alquilo C₁₋₆,
- vii) cicloalquilo,
- viii) cicloalquilo sustituido por 1-4 sustituyentes elegidos individualmente entre
- 30 ciano, ciano-alquilo C₁₋₆, halógeno, halógeno-alcoxi C₁₋₆, halógeno-alquilo C₁₋₆, alcoxi C₁₋₆, alcoxi C₁₋₆-alquilo C₁₋₆ y alquilo C₁₋₆,

R² se elige entre el grupo formado por

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

R³ es alquilo C₁₋₆;

5 R⁴ se elige entre el grupo formado por

- i) hidrógeno y
- ii) alquilo C₁₋₆;

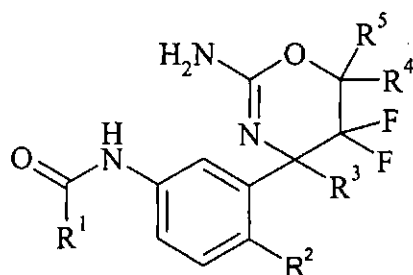
R⁵ se elige entre el grupo formado por

- i) hidrógeno y
- 10 ii) alquilo C₁₋₆;

n es el número 0, 1 ó 2;

o sus sales farmacéuticamente aceptables.

3. Un compuesto de la fórmula la según la reivindicación 1,



1a

15 en la que

R¹ se elige entre el grupo formado por

- i) arilo,
- ii) arilo sustituido por 1-4 sustituyentes elegidos individualmente entre ciano, ciano-alquilo C₁₋₆, halógeno, halógeno-alcoxi C₁₋₆, halógeno-alquilo C₁₋₆,
20 alcoxi C₁₋₆, alcoxi C₁₋₆-alquilo C₁₋₆ y alquilo C₁₋₆,
- iii) heteroarilo y
- iv) heteroarilo sustituido por 1-4 sustituyentes elegidos individualmente entre ciano, ciano-alquilo C₁₋₆, halógeno, halógeno-alcoxi C₁₋₆, halógeno-alquilo C₁₋₆, alcoxi C₁₋₆, alcoxi C₁₋₆-alquilo C₁₋₆ y alquilo C₁₋₆;

25 R² se elige entre el grupo formado por

- i) hidrógeno,
- ii) halógeno y

iii) alquilo C₁₋₆;

R³ es alquilo C₁₋₆;

R⁴ se elige entre el grupo formado por

i) hidrógeno y

5 ii) alquilo C₁₋₆;

R⁵ se elige entre el grupo formado por

i) hidrógeno y

ii) alquilo C₁₋₆;

o sus sales farmacéuticamente aceptables.

10 4. Un compuesto según una cualquiera de las reivindicaciones 1-3, en el que R¹ se elige entre el grupo formado por

i) 1H-pirazolilo no sustituido o sustituido por 1-2 sustituyentes elegidos individualmente entre cicloalquilo, halógeno, halógeno-alquilo C₁₋₆, alquilo C₁₋₆,

15 ii) cicloalquilo no sustituido o sustituido por 1-2 sustituyentes elegidos individualmente entre halógeno y halógeno-alquilo C₁₋₆,

iii) alqueno C₂₋₆ no sustituido o sustituido por heteroarilo,

iv) alquilo C₁₋₆ no sustituido o sustituido por 1-5 sustituyentes elegidos individualmente entre halógeno y hidroxilo,

20 v) furilo no sustituido o sustituido por nitro,

vi) isoxazolilo no sustituido o sustituido por 1-2 alquilo C₁₋₆,

vii) oxazolilo no sustituido o sustituido por 1-2 sustituyentes elegidos individualmente entre cicloalquilo, halógeno-alquilo C₁₋₆ y alquilo C₁₋₆,

25 viii) pirazinilo no sustituido o sustituido por 1-2 sustituyentes elegidos individualmente entre cicloalquil-alcoxi C₁₋₆, halógeno, halógeno-alquilo C₁₋₆ y alquilo C₁₋₆,

ix) pirazolilo no sustituido o sustituido por 1-2 sustituyentes elegidos individualmente entre halógeno y alquilo C₁₋₆,

x) piridazinilo no sustituido o sustituido por 1-2 halógeno,

30 xi) piridinilo no sustituido o sustituido por 1-2 sustituyentes elegidos individualmente entre amido, ciano, cicloalquil-alcoxi C₁₋₆, cicloalquil-

alquinilo C_{2-6} , halógeno, halógeno-alquilo C_{1-6} , alquilo C_{1-6} y halógeno-alcoxi C_{1-6} ; y

xii) pirimidinilo no sustituido o sustituido por 1-2 sustituyentes elegidos individualmente entre halógeno y halógeno-alquilo C_{1-6} .

5 5. Un compuesto según una cualquiera de las reivindicaciones 1-4, en el que R^1 se elige entre el grupo formado por

- i) piridinilo,
- ii) pirazolilo,
- iii) pirazinilo,
- 10 iv) pirimidinilo, y
- v) piridinilo sustituido por 1-2 sustituyentes elegidos individualmente entre ciano, halógeno, y halógeno-alquilo C_{1-6} .

6. Un compuesto según una cualquiera de las reivindicaciones 1-5, en el que R^1 se elige entre el grupo formado por

- 15 (2,2,2-trifluoretoksi)-piridin-2-ilo, (ciclopropilmetoxi)-pirazin-2-ilo, (trifluormetil)-pirazin-2-ilo, 1-(difluor-metil)-1H-pirazol-3-ilo, 1-(trifluormetil)cicloprop-1-ilo, 1-furil-etenilo, 1-metil-1H-pirazol-3-ilo, 2-(clorometil)-oxazol-4-ilo, 2-(fluormetil)oxazol-4-ilo, 2,2,2-trifluor-1-hidroxi-1-metil-2-etilo, 2,2-difluorcicloprop-1-ilo, 2,5-dimetiloxazol-4-ilo, 2-etiloxazol-4-ilo, 2-metil-5-(trifluor-metil)oxazol-4-ilo, 2-metiloxazol-4-ilo, 3-(2,2,2-
- 20 trifluor-etoxi)-piridin-2-ilo, 3,5-dicloropirazin-2-ilo, 3,5-dicloro-piridin-2-ilo, 3,5-difluor-piridin-2-ilo, 3-cloro-5-ciano-piridin-2-ilo, 3-cloro-5-fluor-piridin-2-ilo, 3-cloro-5-trifluormetil-piridin-2-ilo, 3-cloro-piridin-2-ilo, 3-fluor-piridin-2-ilo, 3-trifluormetil-piridin-2-ilo, 4-cloro-1-(2,2,2-trifluoretil)-1H-pirazol-3-ilo, 4-cloro-1-(2,2-di-fluoretil)-1H-pirazol-3-ilo, 4-cloro-1-difluormetil-1H-pirazol-3-ilo, 4-cloro-1-etil-1H-pirazol-3-ilo, 4-cloro-1H-
- 25 pirazol-5-ilo, 4-cloro-1-metil-1H-pirazol-3-ilo, 4-cloro-3-ciclopropil-1H-pirazol-5-ilo, 4-metil-1H-pirazol-5-ilo, 4-metil-isoxazol-3-ilo, 5-(2,2,2-trifluor-etoxi)-piridin-2-ilo, 5-(2,2,3,3,3-pentafluorpropoxi)-piridin-2-ilo, 5-(2,2,3,3-tetrafluorpropoxi)-piridin-2-ilo, 5-(2,2-difluoretoksi)-piridin-2-ilo, 5-(ciclopropiletinil)-piridin-2-ilo, 5-(ciclopropilmetoxi)pirazin-2-ilo, 5-(difluormetoxi)-piridin-2-ilo, 5-(fluormetoxi)-piridin-2-ilo, 5-
- 30 (trifluormetil)-piridin-2-ilo, 5-amido-piridin-2-ilo, 5-cloro-3-fluor-piridin-2-ilo, 5-cloro-3-metil-piridin-2-ilo, 5-cloropirazin-2-ilo, 5-cloro-piridin-2-ilo, 5-cloro-pirimidin-2-ilo, 5-ciano-piridin-2-ilo-1-óxido, 5-ciano-piridin-2-ilo, 5-ciclopropil-oxazol-4-ilo, 5-etil-oxazol-

4-ilo, 5-fluormetoxi-piridin-2-ilo, 5-fluor-piridin-2-ilo, 5-isopropil-oxazol-4-ilo, 5-metil-pirazin-2-ilo, 5-nitro-fur-2-ilo, 5-trifluormetil-pirazin-2-ilo, 5-trifluormetil-pirimidin-2-ilo, 6-(ciclopropilmetoxi)-piridin-3-ilo, 6-cloropiridazin-3-ilo, fur-2-ilo, metilo, oxazolilo y piridin-2-ilo.

5 7. Un compuesto según una cualquiera de las reivindicaciones 1-6, en el que R^1 se elige entre el grupo formado por 5-cloro-piridin-2-ilo, 3-cloro-5-trifluormetil-piridin-2-ilo, 3-cloro-5-fluor-piridin-2-ilo, 3,5-dicloro-piridin-2-ilo, 5-ciano-piridin-2-ilo, 5-cloro-3-fluor-piridin-2-ilo, 5-cloro-piridin-2-ilo y 3-cloro-5-trifluormetil-piridin-2-ilo.

8. Un compuesto según una cualquiera de las reivindicaciones de 1 a 7,
10 en el que n es el número 1.

9. Un compuesto según una cualquiera de las reivindicaciones de 1 a 8, en el que R^2 es halógeno.

10. Un compuesto según una cualquiera de las reivindicaciones de 1 a 9, en el que R^2 es flúor.

15 11. Un compuesto según una cualquiera de las reivindicaciones de 1 a 10, en el que R^3 es metilo.

12. Un compuesto según una cualquiera de las reivindicaciones de 1 a 11, en el que R^4 es hidrógeno.

13. Un compuesto según una cualquiera de las reivindicaciones de 1 a 12,
20 en el que R^4 es metilo.

14. Un compuesto según una cualquiera de las reivindicaciones de 1 a 13, en el que R^5 es hidrógeno.

15. Un compuesto según una cualquiera de las reivindicaciones de 1 a 14, en el que R^5 es metilo.

25 16. Un compuesto según una cualquiera de las reivindicaciones de 1 a 15, elegido entre el grupo formado por

[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-piridina-2-carboxílico,

30 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-fluor-piridina-2-carboxílico,

[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-piridina-2-carboxílico,

- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3,5-difluor-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-5-trifluormetil-piridina-2-carboxílico,
- 5 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-trifluormetil-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-5-fluor-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida
- 10 del ácido 3,5-dicloro-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-fluor-piridina-2-carboxílico,
- 15 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-ciano-piridina-2-carboxílico,
- clorhidrato de la [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-ciano-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida
- 20 del ácido 5-ciano-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-pirimidina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-3-metil-piridina-2-carboxílico,
- 25 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-(2,2,2-tri-fluor-etoxi)-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-3-fluor-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida
- 30 del ácido 5-trifluormetil-pirimidina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-trifluormetil-pirazina-2-carboxílico,

- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 4-cloro-1-metil-1H-pirazol-3-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-piridina-2-carboxílico,
- 5 [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-5-trifluormetil-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-ciano-piridina-2-carboxílico,
- clorhidrato de la (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-3-cloro-5-cianopicolinamida,
- 10 (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-3-cloro-5-cianopicolinamida,
- clorhidrato de la (R)-N-(3-(2-amino-5,5-difluor-4,6,6-tri-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-3,5-dicloropicolinamida,
- 15 (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-3,5-dicloropicolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-cloro-3-fluorpicolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(trifluormetil)picolinamida,
- 20 (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(fluormetoxi)picolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(difluormetoxi)picolinamida,
- 25 (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(2,2-difluoretoxi)-picolinamida,
- [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-(2,2,2-trifluor-etoxi)-piridina-2-carboxílico,
- clorhidrato de la [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-(2,2,3,3-tetrafluor-propoxi)-piridina-2-carboxílico,
- 30 [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-(2,2,3,3-tetrafluor-propoxi)-piridina-2-carboxílico,

- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-6-(ciclopropilmetoxi)-nicotinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-cloropirimidina-2-carboxamida,
- 5 [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-trifluor-metil-pirimidina-2-carboxílico,
- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-metilpirazina-2-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-10 5-(trifluormetil)pirazina-2-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(ciclopropilmetoxi)pirazina-2-carboxamida,
- clorhidrato de la (R)-N-(3-(2-amino-5,5-difluor-4,6,6-tri-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-6-cloropiridazina-3-carboxamida,
- 15 (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-6-cloropiridazina-3-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-1-(difluormetil)-1H-pirazol-3-carboxamida,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida
- 20 del ácido 3-cloro-5-ciano-piridina-2-carboxílico,
- (S)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(ciclopropiletinil)picolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(difluormetoxi)picolinamida,
- 25 (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(fluormetoxi)picolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(2,2,3,3-tetrafluorpropoxi)-picolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-
- 30 (2,2,3,3,3-pentafluorpropoxi)-picolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(2,2-difluoretoxi)picolinamida,

- (S)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-(ciclopropilmetoxi)pirazina-2-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-cloropirazina-2-carboxamida,
- 5 (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-3,5-dicloropirazina-2-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4,5-difluorfenil)-5-cianopicolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-clorofenil)-5-
- 10 cloropicolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-clorofenil)-5-cianopicolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)fenil)-5-cloropicolinamida,
- 15 (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)fenil)-5-cianopicolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-3-(2,2,2-trifluoretoxi)-picolinamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-
- 20 fluorfenil)oxazol-4-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-2-etiloxazol-4-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-2-(clorometil)oxazol-4-carboxamida,
- 25 (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-2-metiloxazol-4-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-2,5-dimetiloxazol-4-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-2-
- 30 metil-5-(trifluormetil)oxazol-4-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-4-metilisoxazol-3-carboxamida,

- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-isopropiloxazol-4-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-1-metil-1H-pirazol-3-carboxamida,
- 5 (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-1-(difluormetil)-1H-pirazol-3-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-4-cloro-1H-pirazol-5-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-4-
- 10 metil-1H-pirazol-5-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-4-cloro-3-ciclopropil-1H-pirazol-5-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-4-cloro-1-(2,2-difluoretil)-1H-pirazol-3-carboxamida,
- 15 formiato de la (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-etiloxazol-4-carboxamida,
- formiato de la (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-5-ciclopropil-oxazol-4-carboxamida,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida
- 20 del ácido 4-cloro-1-difluormetil-1H-pirazol-3-carboxílico,
- (R)-N2-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)piridina-2,5-dicarboxamida,
- N-[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-acetamida,
- 25 N-(3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-2,2-difluorciclopropano-carboxamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-1-(trifluormetil)ciclopropano-carboxamida,
- 2,2,2-trifluoracetato de la (R)-N-(3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-
- 30 oxazin-4-il)-4-fluor-fenil)-3,3,3-trifluor-2-hidroxi-2-metilpropanamida,
- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-4-cloro-1-etil-1H-pirazol-3-carboxamida,

- (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-4-cloro-1-(2,2,2-trifluoretil)-1H-pirazol-3-carboxamida,
 formiato de la (R)-N-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenil)-2-(fluormetil)-oxazol-4-carboxamida,
- 5 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido furano-2-carboxílico,
 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-nitro-furano-2-carboxílico,
 (E)-N-[3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-
 10 3-furan-2-il-acrilamida y
 (R)-2-(3-(2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-1,3-oxazin-4-il)-4-fluorfenilcarbamoil)-5-cianopiridina 1-óxido,
 o una sal farmacéuticamente aceptable de los mismos.
17. Un compuesto según una cualquiera de las reivindicaciones de 1 a 16,
- 15 elegido entre el grupo formado por
 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-piridina-2-carboxílico,
 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-fluor-piridina-2-carboxílico,
 20 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-piridina-2-carboxílico,
 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3,5-difluor-piridina-2-carboxílico,
 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida
 25 del ácido 3-cloro-5-tri-fluormetil-piridina-2-carboxílico,
 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-trifluormetil-piridina-2-carboxílico,
 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-5-fluor-piridina-2-carboxílico,
 30 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3,5-dicloro-piridina-2-carboxílico,

- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-fluor-piridina-2-carboxílico,
- 5 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-ciano-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-pirimidina-2-carboxílico,
- 10 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-3-metil-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-(2,2,2-tri-fluor-etoxi)-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-3-fluor-piridina-2-carboxílico,
- 15 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-trifluormetil-pirimidina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-trifluormetil-pirazina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 4-cloro-1-metil-1H-pirazol-3-carboxílico,
- 20 [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-piridina-2-carboxílico y
- [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-5-trifluormetil-piridina-2-carboxílico.
- 25 18. Un compuesto según una cualquiera de las reivindicaciones de 1 a 10, elegido entre el grupo formado por
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-5-tri-fluormetil-piridina-2-carboxílico,
- 30 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3-cloro-5-fluor-piridina-2-carboxílico,

- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 3,5-dicloro-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-ciano-piridina-2-carboxílico,
- 5 [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]-oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-3-fluor-piridina-2-carboxílico,
- [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-cloro-piridina-2-carboxílico y
- [3-((R)-2-amino-5,5-difluor-4,6,6-trimetil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-
- 10 amida del ácido 3-cloro-5-trifluormetil-piridina-2-carboxílico.

19. Un compuesto según una cualquiera de las reivindicaciones de 1 a 18, el cual es clorhidrato de la [3-((R)-2-amino-5,5-difluor-4-metil-5,6-dihidro-4H-[1,3]oxazin-4-il)-4-fluor-fenil]-amida del ácido 5-ciano-piridina-2-carboxílico.

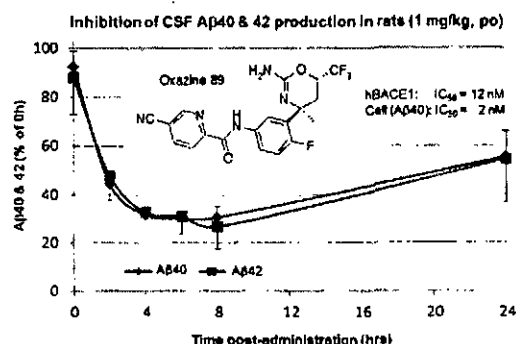
β -Secretase (BACE1) Inhibitors with High in Vivo Efficacy Suitable for Clinical Evaluation in Alzheimer's Disease

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 Kuglstatter,[§] 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

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.

Received: February 13, 2013

Published: April 16, 2013

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

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

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

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

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

CHEMISTRY

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

The ester group in 4 and 5 was then reduced to the alcohols 6 and 7 with LiBH₄, which was followed by cleavage of the chiral auxiliary under acidic conditions to give the amino alcohols 8 and 9.³² Cyclization of the amino alcohols 8 and 9 was readily accomplished with cyanogen bromide to give the oxazines 10 and 11, which were reduced to the corresponding anilines 12³³ and 13 by catalytic hydrogenation. Amide formation was accomplished without prior protection of the amidine moiety using 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholinium chloride hydrate,³⁴ furnishing the target compounds 1a–b and 14a–v (Table 2). The absolute configurations were assigned based on X-ray structures of 1b and 14d complexed to BACE1.

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

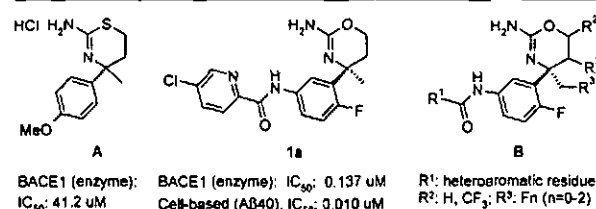
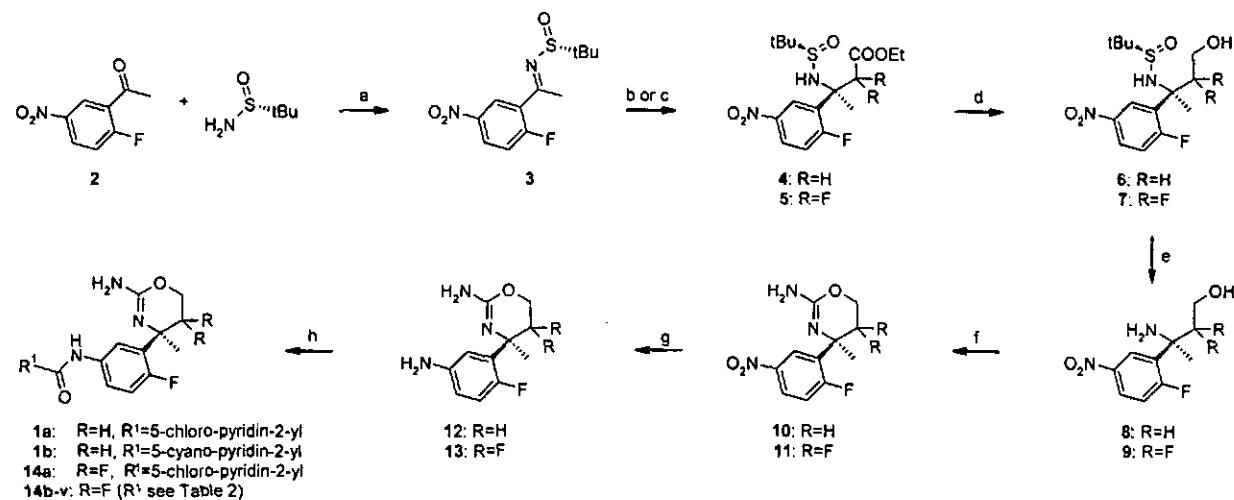


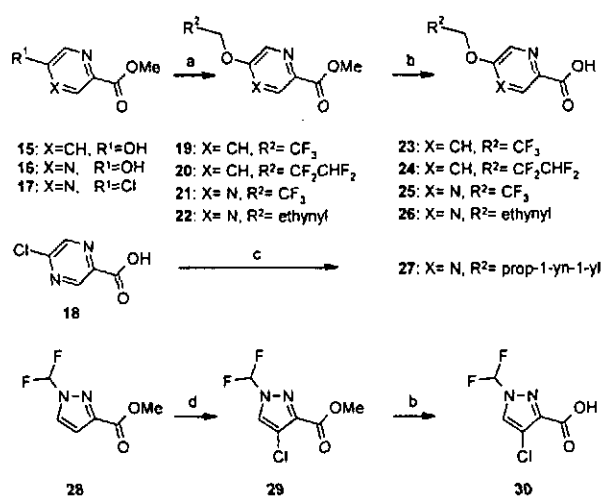
Figure 1. Lead compound 1a and targeted fluoro-oxazines B.

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

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

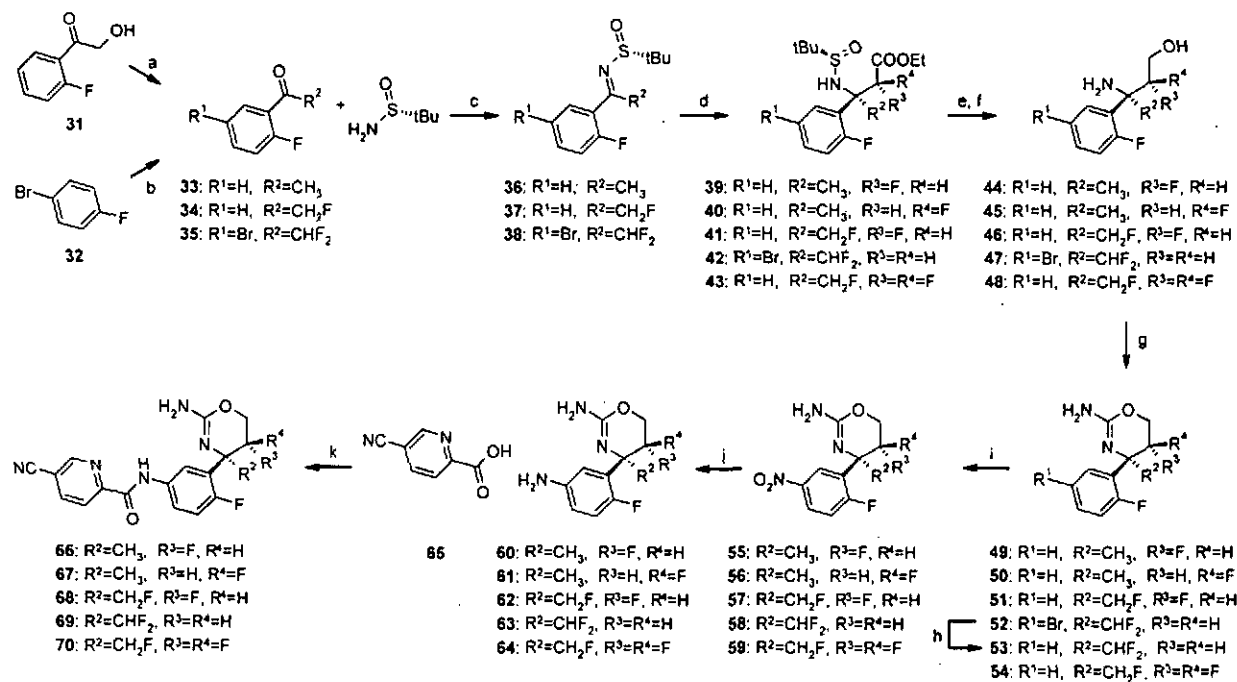
^aReagents and conditions: (a) 15→19: CF₃CH₂OTf, NaH, DMF, 22 °C. 15→20: CHF₂CF₂CH₂OTf, K₂CO₃, acetone. 17→21: CF₃CH₂OH, Cs₂CO₃, DMF. 17→22: prop-2-yn-1-ol, *tert*-BuOK, 1,4-dioxane, 22 °C. (b) LiOH or NaOH, THF/H₂O, 22 °C to reflux. (c) But-2-yn-1-ol, *tert*-BuOK, DMF, 65 °C. (d) N-chlorosuccinimide, 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).

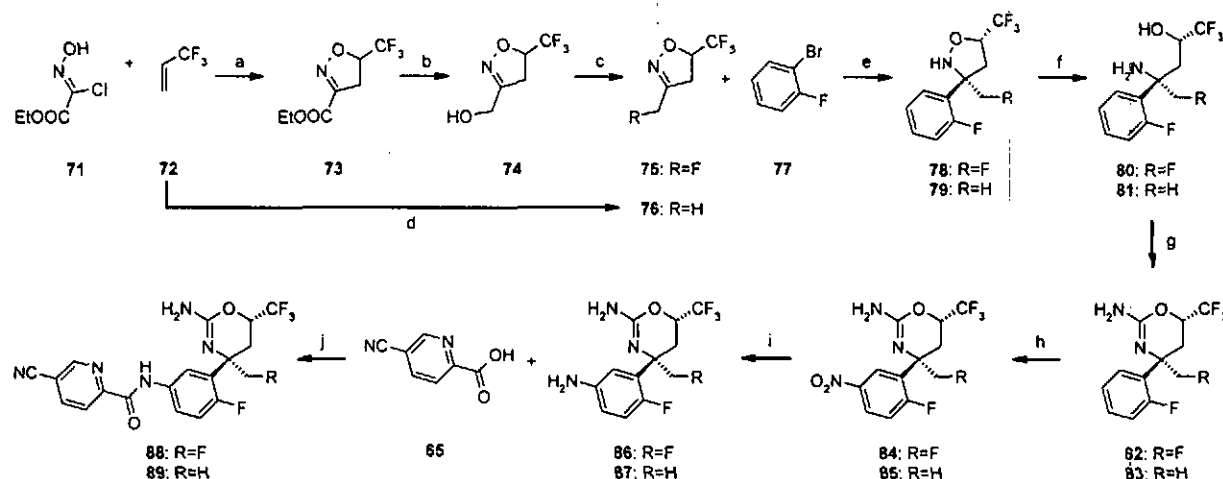
Compound 27 was prepared directly from the acid 18 without proceeding via the corresponding ester. Finally, chlorination of the pyrazole ester 28 with *N*-chlorosuccinimide followed by saponification delivered the chloro-pyrazole derivative 30.

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 BrCHFCOOEt yielded a separable 4:1-mixture of the epimers 39 and 40. Conversion of 37 with BrCHFCOOEt gave a separable 8:1-mixture of 41 as the major isomer and the corresponding epimer (R³=H, R⁴=F). Oxazines 49–51 and 53–54, prepared in analogy to Scheme 1, were then nitrated with fuming nitric acid, followed by reduction of the nitro group and amide formation in the usual manner, to give the targeted fluorinated oxazines 66–70. The relative and absolute configurations were assigned based on X-ray structures of 66–70 complexed to BACE1.

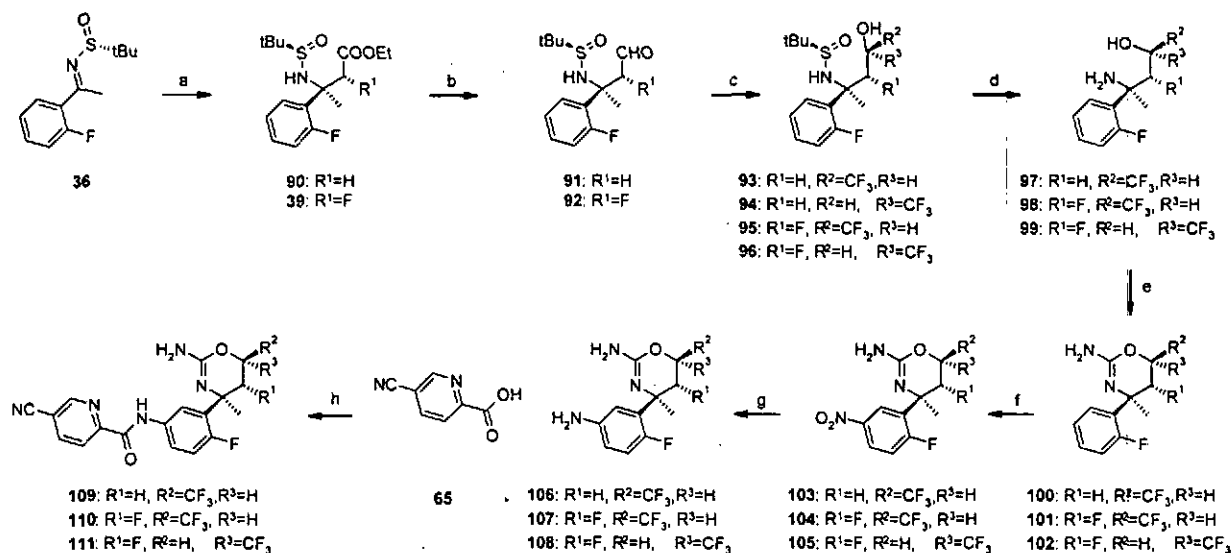
The synthesis of trifluoromethyl substituted oxazines 88 and 89 (Scheme 4) required a different strategy. Ready access to CF₃ substituted 3-amino-propan-1-ol derivatives 80 and 81 is achieved using a dipolar cycloaddition reaction of a nitrile oxide

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

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

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

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

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

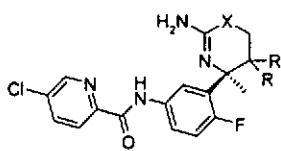
^aReagents and conditions: (a) 36→39: BrCHFCOOEt, Zn, CuCl, THF. 36→90: BrCH₂COOEt, Zn, CuCl, THF. (b) DIBAH, toluene, -76 °C. (c) Trimethyl(trifluoromethyl)silane, THF, TBAF, 0 °C. (d) aq HCl, THF, 22 °C. (e) BrCN, EtOH, 85 °C. (f) H₂SO₄, HNO₃, 0 °C. (g) H₂, Pd/C, EtOH, Et₃N, 22 °C. (h) DMTMM, MeOH,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

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

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

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

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



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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

		R ¹ =								
				14a	14b	14c	14d	14e	14f	14g
				14h	14i	14j	14k	14l	14m	14n
				14o	14p	14q	14r	14s	14t	14u
				14v						

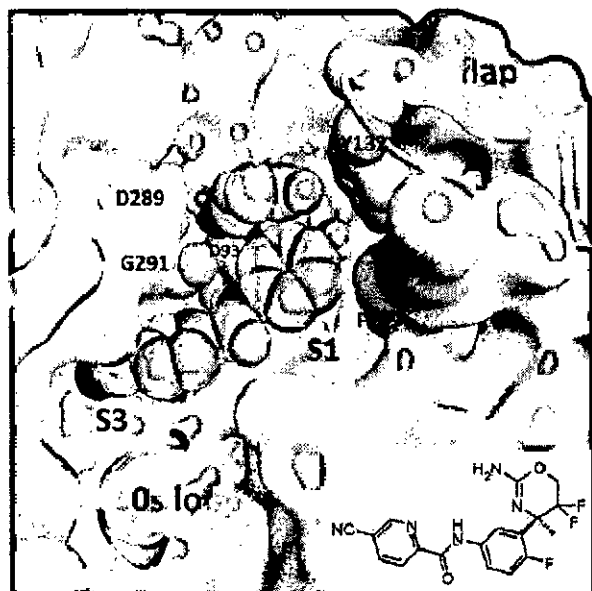


Figure 2. 14d bound to the BACE1 binding site. The amidine recognition motif is tightly bound to the catalytic aspartates D289 and D93 (partially hidden) in the bottom of the pocket. The fluorine atoms in the headgroup and the *o*-F substituent of the phenyl ring (marked in green) form a network of vdW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



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

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

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

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

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

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

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

Chemical structures of compounds 1b, 66, 67, 69, 109, 89, 68, 88, 111, 110, 14d, and 70 are shown. The structures are oxazine derivatives with various fluorine and trifluoromethyl substituents.

IC ₅₀ (μM) ^a											
compd	pK _a ^b	ΔpK _a to 1b	log D ^c	log P	Δlog D/log P	hBACE1 enzyme	Aβ40 cell-based ^d	shift BACE1 enzyme vs cell- based	P-gp ER ^e	hERG inhibition at 10 μM (%)	brain Aβ40 reduction in mice at 10/3/1 mg/kg (%)
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	

^aIC₅₀ values are means of at least two independent experiments. ^bpK_a determined by capillary electrophoresis. ^clog 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.

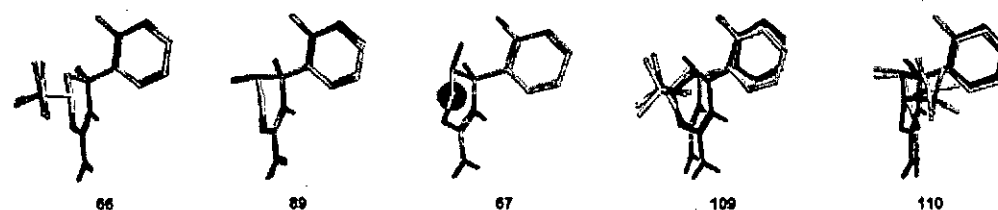


Figure 4. X-ray structures (yellow) of fluorinated head groups with the S1 phenyl ring were energy minimized in the unbound state by a semiempirical geometry optimization with the PM3 Hamiltonian (magenta). The coordinates of 66 and 89 remain almost unchanged as compared to the bound conformation. Therefore, 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 70³¹) 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 CF₃ group showed increased lipophilicity (log P = 2.9–3.1 in 88, 89, 109–111) compared to 1b. As expected, the Δlog 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 CF₃

group in 89 improved the binding affinity considerably (IC₅₀ = 0.012 μM) compared to unsubstituted 1b (IC₅₀ = 0.051 μM). According to the X-ray structure, the CF₃ 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 CF₃ 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)

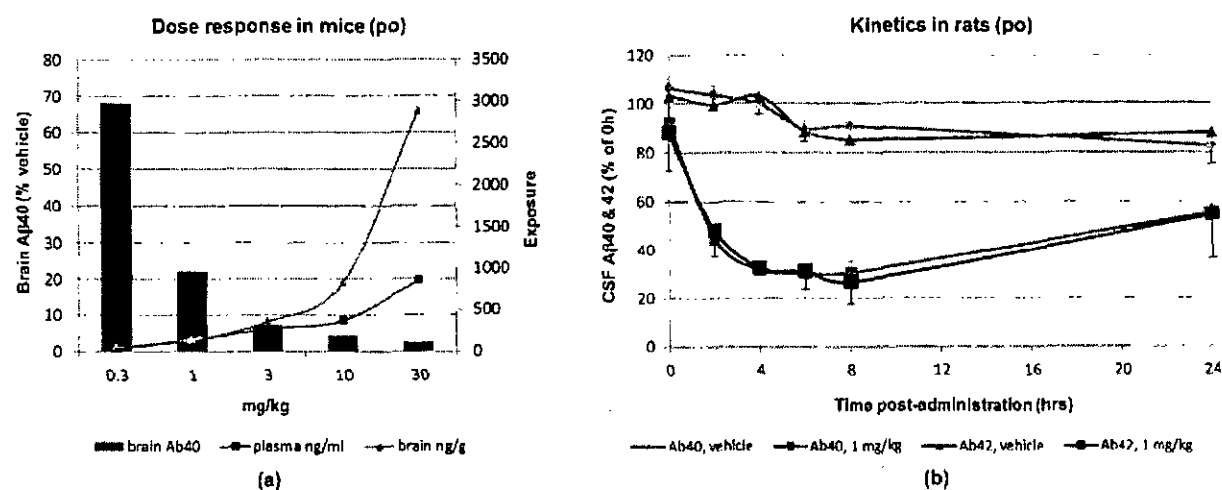


Figure 5. (a) Dose dependent inhibition of brain Aβ40 production in mice by compound 89 determined 4 h oral post dose, $n = 3-4$, mean reduction as compared to vehicle control. (b) Time dependent inhibition of CSF Aβ40/42 production in rats after an oral dose of 1 mg/kg of compound 89, $n = 2-3$. CSF 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 μM .

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 0.010 μM) was lower compared to 66 ($IC_{50} = 0.001$ μM) 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 $IC_{50} = 0.002$ μM , P-gp efflux of 1.9) was most potent showing a reduction of Aβ40 of 78% at a dose as low as 1 mg/kg.

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

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

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

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

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

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

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

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

SUMMARY AND CONCLUSION

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

EXPERIMENTAL SECTION

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

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

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

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

combined filtrates were subsequently washed with aqueous citric acid (5%) and aqueous saturated NaHCO_3 solution, the organic layer was dried over Na_2SO_4 and filtered, and the filtrate was evaporated. The residue was purified by flash column chromatography on silica gel using a mixture of *n*-heptane/EtOAc (2:1) to give the title compound **5** (17.24 g, 69%) as a yellow oil. ^1H NMR (300 MHz, CDCl_3) δ ppm 8.51 (dd, J = 6.6, 2.7 Hz, 1 H), 8.29 (ddd, J = 9.1, 3.8, 3.0 Hz, 1 H), 7.26 (dd, J = 11.3, 9.1 Hz, 1 H), 4.72 (s, 1 H), 4.34 (qd, J = 7.2, 1.5 Hz, 2 H), 2.09–2.17 (m, 3 H), 1.32 (t, J = 7.3 Hz, 3 H), 1.32 (s, 9 H). MS (ISP): m/z = 411.2 $[\text{M} + \text{H}]^+$. 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-sulfinic Acid [(R)-2,2-Difluoro-1-(2-fluoro-5-nitro-phenyl)-3-hydroxy-1-methyl-propyl]-amide (7).³² To a solution of (R)-2,2-difluoro-3-(2-fluoro-5-nitro-phenyl)-3-((R)-2-methyl-propane-2-sulfinylamino)-butyric acid ethyl ester (**5**) (3.78 g, 9.2 mmol) in dry THF (24 mL) was added at 0 °C lithium borohydride (9.0 mmol), and stirring was continued at 0 °C for 1.5 h. The reaction mixture was quenched by addition of acetic acid (1 mL) and water, the mixture was extracted with EtOAc, and the organic layers were washed with brine, dried over Na_2SO_4 , and evaporated. The residue was purified by flash column chromatography on silica gel using a mixture of *n*-heptane/EtOAc (3:2) to give the title compound **7** (2.90 g, 86%) as a pale-yellow solid. ^1H NMR (600 MHz, CDCl_3) δ ppm 8.46 (dd, J = 6.8, 2.8 Hz, 1 H), 8.25 (ddd, J = 9.0, 3.7, 2.9 Hz, 1 H), 7.25 (dd, J = 11.7, 9.0 Hz, 1 H), 5.36 (br s, 1 H), 4.51–5.03 (br s, 1 H), 4.21 (ddd, J = 25.0, 13.4, 2.7 Hz, 1 H), 3.84 (td, J = 12.8, 7.9 Hz, 1 H), 2.19 (s, 3 H), 1.31 (s, 9 H). MS (ISP): m/z = 369.0 $[\text{M} + \text{H}]^+$. A small sample was recrystallized from EtOAc/*n*-heptane: mp 154–155 °C. Anal. ($\text{C}_{14}\text{H}_{19}\text{F}_3\text{N}_3\text{O}_5\text{S}$) Calcd: C 45.65%, H 5.20%, N 16.21%, S 8.70%. Found: C 45.60%, H 5.07%, N 16.34%, S 8.78%.

(R)-3-Amino-2,2-difluoro-3-(2-fluoro-5-nitro-phenyl)-butan-1-ol (9).³² A solution of (R)-2-methyl-propane-2-sulfinic acid [(R)-2,2-difluoro-1-(2-fluoro-5-nitro-phenyl)-3-hydroxy-1-methyl-propyl]-amide (**7**) (3.79 g, 10.3 mmol) in MeOH (50 mL) was treated with a solution of HCl in 1,4-dioxane (4 M, 13 mL), and stirring was continued at 22 °C for 1 h. The mixture was evaporated, the residue was partitioned between EtOAc and aqueous Na_2CO_3 solution (2 M), and the organic layers were washed with brine, dried over Na_2SO_4 , filtered, and evaporated to give the crude title compound **9** (2.50 g, 92%) as a pale-yellow solid, which was used without further purification. ^1H NMR (600 MHz, CDCl_3) δ ppm 8.49 (dd, J = 7.0, 2.8 Hz, 1 H), 8.25 (ddd, J = 9.0, 3.8, 2.9 Hz, 1 H), 7.25 (dd, J = 11.5, 9.0 Hz, 1 H), 4.02 (ddd, J = 19.7, 12.7, 8.6 Hz, 1 H), 3.86 (dd, J = 23.5, 11.8 Hz, 1 H), 2.01–3.63 (3 H), 1.85 (d, J = 0.9 Hz, 3 H). MS (ISP): m/z = 265.1 $[\text{M} + \text{H}]^+$. A small sample was triturated with 2-propanol: mp 110–111 °C. Anal. ($\text{C}_{10}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_3$) Calcd: C 45.46%, H 4.20%, N 10.60%. Found: C 45.67%, H 4.20%, N 11.03%.

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

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

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

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

5-Cyano-pyridine-2-carboxylic Acid [3-((R)-2-Amino-5,5-difluoro-4-methyl-5,6-dihydro-4H-[1,3]oxazin-4-yl)-4-fluoro-phenyl]-amide (14d).⁴³ The condensation of **13** (80 mg, 0.31 mmol) and 5-cyano-pyridine-2-carboxylic acid (50 mg, 0.34 mmol) yielded the title compound **14d** (78 mg, 65%) as a colorless solid. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ ppm 10.86 (s, 1 H), 9.20 (dd, J = 2.1, 0.9 Hz, 1 H), 8.58 (dd, J = 8.2, 2.1 Hz, 1 H), 8.28 (dd, J = 8.2, 0.8 Hz, 1 H), 7.98 (dd, J = 7.2, 2.8 Hz, 1 H), 7.88 (dd, 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 $\text{C}_{16}\text{H}_{13}\text{F}_3\text{N}_4\text{O}_2$, 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, $J = 12.4, 6.0, 6.0, 6.0, 2.8$ 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, $J = 12.3, 6.0, 6.0, 6.0, 2.8$ Hz, 1 H), 3.90–4.60 (br s, 2 H), 3.57 (br s, 2 H), 2.76 (dd, $J = 13.5, 2.8$ Hz, 1 H), 1.81 (dd, $J = 13.4, 12.6$ Hz, 1 H), 1.60 (d, $J = 1.2$ Hz, 3 H). MS: $m/z = 292.5$ [$M + H$] $^+$. Anal. ($C_{12}H_{13}F_4N_2O$) Calcd: C 49.49%, H 4.50%, N 14.43%. Found: C 49.25%, H 4.51%, N 14.15%.

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

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

the assay was prepared and purified as described.⁵⁸ The preparation of BACE2 was carried out following exactly the known protocol.⁴⁶ BACE1/2 assay: The final enzyme concentration in the assay was 30 and 125 nM, respectively. The assay buffer was 100 mM sodium acetate, 20 mM EDTA, 0.05% BSA, pH 4.0. The substrate peptide was of sequence WSEVNLDAEFRMR121 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 Aβ₄₀ concentrations by AlphaLISA technique using PerkinElmer Human Amyloid β 1–40 kit (Aβ1–40, high specificity, cat. no. AL275 C/P). In a PerkinElmer OptiPlate -384 (cat. no. 6007290), 6 μL of culture supernatants were added to 2 μL of AlphaLISA Anti hu Aβ acceptor beads (50 μg/mL), 5 nM Biotinylated Antibody Anti Aβ1–40, 1× AlphaLISA HiBlock buffer solution and incubated for 1 h at room temperature. Then 16 μL 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 Aβ₄₀ in the culture supernatants were calculated as percentage of maximum signal (cells treated with 1% DMSO without inhibitor). The IC₅₀ values were calculated using the Excel XLfit software.

P-gp Assay. Bidirectional transport was examined in LLC-PK1 cells (Lewis-lung cancer porcine kidney 1 L, ATCC CL-101) stably expressing human MDR1. The cell lines were obtained from Dr. Alfred Schinkel, The Netherlands Cancer Institute (Amsterdam, The Netherlands) and used under a license agreement. Both cell lines were cultured on semipermeable insert plates (Costar, surface area 0.33 cm², pore size 3.0 μm), 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 μM), and the cell monolayer integrity was assessed via the permeability of lucifer yellow (10 μM). 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 μM) 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 Aβ₄₀ 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 Aβ₄₀ and plasma was used for determination of compound exposure. The method for Aβ₄₀ determination in brain lysates followed the known procedure.⁶¹ Brain tissue was homogenized in 2% DEA buffer in a Roche MagnaLyser (20", 4000 rpm) and subsequently centrifuged for 1 h at 10000g. DEA was reduced to 0.2% in 50 mM NaCl and one-half of the DEA lysate was passed over an Oasis Solid phase extraction plate (Waters; cat. no. 186000679), which had been activated with MeOH and equilibrated in dH₂O (1 mL each). After washes in 10% and 30% MeOH (1 mL each), the Aβ-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 μL of AlphaLISA assay buffer. Aβ₄₀ was determined by an AlphaLISA assay (Perkin-Elmer). In a white 96-well, half area microplate (Perkin-Elmer cat. no. 6005561), 20 μL of the reconstituted sample were mixed with 5 μL of biotinylated BAP-24 (specific for C-terminus of Aβ₄₀⁶²), stock = 4.4 mg/mL, f.c.5.5 μg/mL, and 5 μL 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 μL 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 Aβ₄₀ in Wild-Type Rats. Animals and Housing Conditions. Animals were maintained in a 12:12 h

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

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

■ ASSOCIATED CONTENT

■ Supporting Information

Preparation details and analytical data for compounds 1a–b, 14b–c, 14e–v, 66–70, 88, 109–113. Log *D*, p*K*_a determinations, stability in mouse microsomes, inhibition of CYP_{450a} and time dependent inhibition of CYP₄₅₀ 3A4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Accession Codes

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

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +41 61 688 72 64. E-mail: hans.hilpert@roche.com. Address: F. Hoffmann-La Roche Ltd, Bldg. 92/2.88, CH-4070 Basel, Switzerland.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank A. Fettes, R. Halm, C. Kuratli, and H. P. Marty for synthesis support, D. Zimmerli for separations on chiral HPLC, J. Schneider for NMR spectroscopy, C. Bartelmus for mass spectroscopy, L. Polonchuk for hERG data, S. Bendels for log *P* calculations, M. Stühle and D. Schlatter for protein crystallization and production, and N. Kratochwil, A. Pähler, A. Poirier, V. Starke, and K. Youdim for DMPK support.

■ 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, L.; 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.; Suomensaari, 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.; Loebloff, 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, S33–S37.
- (19) Lin, X.; Koelsch, G.; Wu, S.; Downs, D.; Dashti, A.; Tang, J. Human aspartic 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) Kurishima, 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, I.; 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 β 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.; Gregor, A. R.; Steinbeiser, M. A.; Shaffer, J. R.; Nantermet, P. G.; Barrow, J. C.; Rittle, K. E.; Collisi, 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.; Kuglstatter, A.; Kuszniir, 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.; Staufenberg, 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) Kuglstatler, 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.