Journal of
Medicinal Chemistry

Subscriber access provided by UNIV OF NEW ENGLAND ARMIDALE

Brief Article

Discovery of an Extremely Potent Thiazine-Based #-Secretase (BACE1) Inhibitor with Reduced Cardiovascular and Liver Toxicity at a Low Projected Human Dose

Genta Tadano, Kazuo Komano, Shuhei Yoshida, Shinji Suzuki, Kenji Nakahara, Kouki Fuchino, Kazuki Fujimoto, Eriko Matsuoka, Takahiko Yamamoto, Naoya Asada, Hisanori Ito, Gaku Sakaguchi, Naoki Kanegawa, Yasuto Kido, Shigeru Ando, Tamio Fukushima, Ard Teisman, Vijay Urmaliya, Deborah Dhuyvetter, Herman Borghys, An Van Den Bergh, Nigel Austin, Harrie J.M. Gijsen, Yoshinori Yamano, Yasuyoshi Iso, and Ken-ichi Kusakabe

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b01140 • Publication Date (Web): 24 Sep 2019

Downloaded from pubs.acs.org on September 24, 2019

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

	Gijsen, Harrie; Janssen Research & Development, Janssen Pharmaceutica NV, Neuroscience Medicinal Chemistry Yamano, Yoshinori; Shionogi and Co Ltd Pharmaceutical Research Center , Discovery Research Laboratory for Core Therapeutic Areas Iso, Yasuyoshi; Shionogi and Co Ltd Pharmaceutical Research Center , Medicinal Chemistry Kusakabe, Ken-ichi; Shionogi and Co Ltd Pharmaceutical Research Center , Medicinal Chemistry

SCHOLARONE™
Manuscripts

Discovery of an Extremely Potent Thiazine-Based β-Secretase (BACE1) Inhibitor with Reduced Cardiovascular and Liver Toxicity at a Low Projected Human Dose

Genta Tadano,[†] Kazuo Komano,[†] Shuhei Yoshida,[†] Shinji Suzuki,[†] Kenji Nakahara,[†] Kouki Fuchino,[†] Kazuki Fujimoto,[†] Eriko Matsuoka,[†] Takahiko Yamamoto,[†,#] Naoya Asada,[†] Hisanori Ito,[†] Gaku Sakaguchi,[†] Naoki Kanegawa,[‡] Yasuto Kido,[‡] Shigeru Ando,[‡] Tamio Fukushima,[‡] Ard Teisman,[§] Vijay Urmaliya,[§] Deborah Dhuyvetter,[§] Herman Borghys,[§] An Van Den Bergh,[∥] Nigel Austin,[∥,◊] Harrie J. M. Gijsen,[∥] Yoshinori Yamano,[†] Yasuyoshi Iso,[†] and Ken-ichi Kusakabe[*,†]

[†]Discovery Research Laboratory for Core Therapeutic Areas, [‡]Research Laboratory for Development, Shionogi Pharmaceutical Research Center, 1-1 Futaba-cho 3-chome, Toyonaka, Osaka 561-0825, Japan

[§]Non-Clinical Safety, [∥]Discovery Sciences, Janssen Research & Development, Turnhoutseweg 30, B-2340 Beerse, Belgium

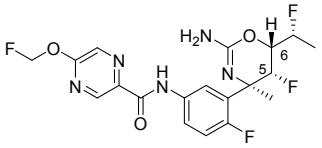
ABSTRACT: Genetic evidence points to deposition of amyloid-β (Aβ) as a causal factor for Alzheimer's disease. Aβ generation is initiated when β-secretase (BACE1) cleaves the amyloid precursor protein. Starting with an oxazine lead **1**, we describe the discovery of a thiazine-based BACE1 inhibitor **5** with robust Aβ reduction in vivo at low concentrations, leading to a low projected human dose of 14 mg/day where **5** achieved sustained Aβ reduction of 80% at trough level.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and constitutes a massive health care burden.[1] Genetic evidence of autosomal dominant AD mutations in the amyloid precursor protein (APP) and presenilin genes provides a support for the Aβ hypothesis that accumulation of amyloid-β (Aβ) could be a causative factor of AD.[2] The Icelandic mutation observed in APP, A673T, further supports this hypothesis, where the mutation protects against AD and cognitive impairment in elderly individuals.[3] The β-site amyloid precursor protein cleaving enzyme 1 (BACE1; also known as β-secretase) is a rate-limiting enzyme for the production of Aβ, along with the γ-secretase complex, is one of the prime targets for AD therapeutics.[4] BACE1 inhibitors have been pursued by a number of pharmaceutical companies and academic institutes,[5] culminating in clinical compounds reaching Phase III clinical trials, such as verubecestat (MK-8931; Merck),[6] lanabecestat (AZD-3293; AstraZeneca and Eli Lilly),[7] elenbecestat (E-2609; Eisai and Biogen),[8] umibecestat (CNP-520; Novartis and Amgen),[9] and atabecestat (JNJ-54861911; Janssen and Shionogi).[10] Of these, verubecestat[6c] and lanabecestat[7b] were unfortunately discontinued due to lack of efficacy, and atabecestat failed due to serious elevations of liver enzymes.[10b] Recently, the discontinuation of their clinical development for umibecestat was announced, where it caused decline in cognitive function.[9b]

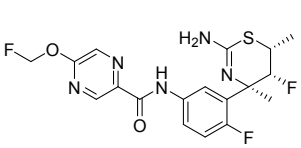
Our research efforts of exploring back-up molecules for atabecestat started in 2011, in order to mitigate the issues observed in preclinical investigations in terms of cardiovascular and liver toxicity, such as a low hERG IC_{50} value and elevations of liver enzymes in high dose in vivo toxicology studies. Thus, our initial objectives were to mitigate these issues along with achieving a low projected human dose and structural differentiation. Our early efforts led to the identification of an oxazine series, such as compound **1** with minimal cardiovascular safety concerns related to hERG inhibition as well as structurally differentiated compounds.[11] The work presented here builds on this series, leading to the discovery of compound **5** with a low human projected dose and no significant concerns on cardiovascular safety and liver toxicity in vivo.

Chart 1. BACE1 Inhibitors 1 and 5



1

BACE1 IC_{50} = 12 nM
Cell Aβ IC_{50} = 1.1 nM
Dog Aβ EC_{50} = 32 ng/mL (73 nM)



5

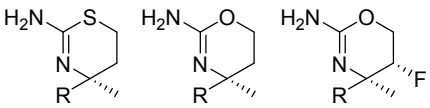
BACE1 IC_{50} = 5.0 nM
Cell Aβ IC_{50} = 0.38 nM
Dog Aβ EC_{50} = 8.9 ng/mL (21 nM)

RESULTS & DISCUSSION

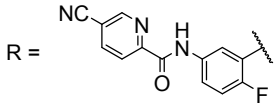
Inhibitor Design. Minimizing the maximum daily dose of a drug candidate is a key strategy to mitigating toxicity risks in clinical applications. Analysis of the top 200 drugs in the United States by Stepan et al. revealed that a maximum daily dose of 100 mg or less significantly reduced the incidence of drugs withdrawn and a black box warning due to idiosyncratic toxicity; more importantly, a daily dose of 10 mg or less led to complete avoidance of the risk.[12] With these points in mind, our medicinal chemistry efforts started with **1** to achieve a low human projected dose. Our SAR efforts related to **1** revealed the following: 1) an optimal pK_a range of 6.5 to 7.4 could avoid high hERG inhibition and P-gp efflux as well as retain cellular potency;[11b] 2) high potency was realized when incorporating substituents at the 5- and 6-positions via active conformation stabilization;[11a] and 3) favorable ADMET profiles, including hERG inhibition, P-gp efflux, and metabolic stability, were

achieved by incorporating fluoromethoxypyrazine.[11] As shown in Table 1, comparative analysis of our early leads **2** and **3**[11a,b] indicated that replacing the oxazine ring in **1** by thiazine[13] leading to **A** could provide further gain in potency (Figure 1), whereas the basicity needed to be fine-tuned further as it was predicted to be below pK_a 6.5 resulting in reduced cellular potency. The pK_a value of the 5-fluoro oxazine **4**[11b] (pK_a = 8.1) as well as the difference in pK_a between thiazine **2** and oxazine **3** (ΔpK_a = 0.8) led to the design of compound **5** with a predicted pK_a value of 7.3 retaining the other key structural features in the lead **1**.

Table 1. pKa and BACE IC_{50} values for the initial leads



	2	**3**	**4**
BACE1 IC_{50} (nM)	9.9	118	48
pK_a	9.0	9.8	8.1



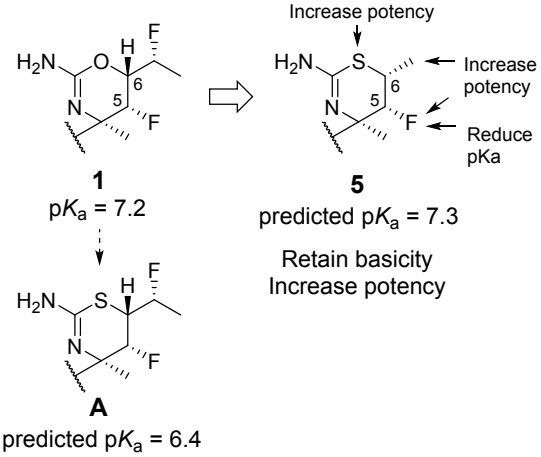
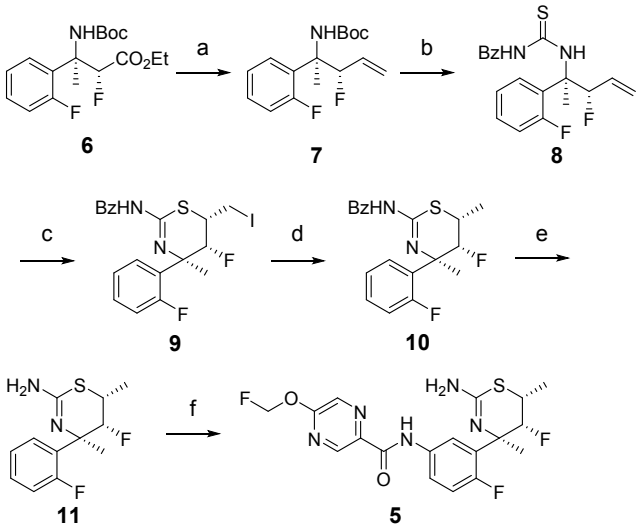


Figure 1. Design of compound **5** from the lead **1**

Chemistry. Synthesis of the thiazine **5** began with treatment of ester **6**[11a] with diisobutylaluminium hydride (DIBAL) followed by Wittig reaction using methyltriphenylphosphonium bromide to give compound **7**. Following deprotection of the Boc group in **7**, the thiazine scaffold was formed with benzoyl isothiocyanate and iodine to afford **9** via intermediate **8**.[11a] Reductive dehalogenation and deprotection of **10** resulted in **11** followed by incorporation of the amide moiety via a three-step sequence allowed access to the final compound **5**.[11]

Scheme 1. Synthesis of compound 5[a]



[a]Reagents and conditions: (a)(i) DIBAL, DCM, -78 °C, (ii) $MePPh_3Br$, KO*t*-Bu, toluene, rt, 39%; (b) (i) HCl in dioxane, DCM, rt, (ii) BzNCS, DCM, rt, 95%; (c) I_2, MeCN, 0 °C, 82%; (d) *n*-Bu_3SnH, AIBN, toluene, 80 °C, 90%; (e) hydrazine hydrate, EtOH, rt, 91%; (f) (i) HNO_3, H_2SO_4-TFA, -20 °C, (ii) Fe, NH_4Cl, toluene-H_2O, 80 °C, (iii) 5-(fluoro methoxy)pyrazine-2-carboxylic acid, HCl, EDC·HCl, MeOH, 0 °C to rt, 91%.

In Vitro and PK Profiling. Compared to the lead **1**, the thiazine **5** was shown to possess a 2- to 3-fold increase in biochemical and cellular potency (BACE1 HTRF IC_{50} = 5.0 nM, cellular $A\beta_{40}$ IC_{50} = 0.38 nM). Compound **5** showed an excellent selectivity over cathepsin D (IC_{50} = >100 μM) and minimal selectivity over BACE2 (HTRF IC_{50} = 7.1 nM). As expected, the designed thiazine **5** exhibited an optimal pK_a value of 7.3, which translated into low hERG inhibitory activity and P-gp recognition in LLC-PK1 cells expressing human MDR1 (hERG inhibition at 3 uM = 31% (IC_{50} = 5.1 μM), P-gp efflux ratio = 1.1). Lipophilicity of **5** was found to be moderate (LogD at pH 7.4 = 2.4). Like compound **1**, thiazine **5** was metabolically stable in human and dog hepatocytes after 2 h incubation at 2 uM (89% and 90% remaining, respectively), whereas in rat, mouse, and cynomolgus monkey, it showed moderate-to-good stability (74%, 71%, and 55%, respectively). In terms of inhibition of CYP enzymes, **5** exhibited no significant inhibition of CYP1A2, CYP2C19, and CYP3A4 (IC_{50} = >20 μM), whereas a moderate inhibition was observed in CYp2C9 and CYP2D6 (IC_{50} = 3.5 μM and 3.3 μM, respectively). The fraction unbound in plasma ($f_{u,p}$) for human, rat, mouse, dog, and monkey was measured to be 0.083, 0.12, 0.15, 0.078, and 0.12, respectively, and the fraction unbound in the rat brain homogenate ($f_{u,b}$) was 0.034. The experimental LogD at pH 7.4 and permeability in LLC-PK1 cells were 2.4 and 22.7 × 10^{-6} cm/s, respectively; aqueous solubility at pH 1.2 and 6.8 was >1000 μg/mL and 28.2 μg/mL, respectively, demonstrating good physicochemical profiles. Reflecting the favorable in vitro ADME profiles, preliminary pharmacokinetic (PK) screening in rat exhibited a low-to-moderate clearance

(24.8 mL/min/kg), a moderate-to-good C_{max} (38.4 ng/mL), an acceptable bioavailability (31%), and high brain-to-total plasma and brain-to-free plasma concentration ratios (K_p = 3.3, $K_{p,uu}$ = 0.95, respectively) at a po dose of 1 mg/kg and iv dose of 0.5 mg/kg. On the basis of the favorable in vitro pharmacological as well as ADME profiles, compound **5** was selected for pharmacokinetic/pharmacodynamic (PK/PD) characterization.

PK/PD Study. The PK/PD profile of **5** was characterized in mouse and dog.[14] In mouse, **5** showed dose-dependent inhibitions of Aβ at 1 and 3 mg/kg (n = 4), where total Aβ in the brain was measured at 2, 4, and 6 h post dosing (Figure 2). Compound **5** reached maximal Aβ reduction of 90% at 3 mg/kg and reduced Aβ by 55% at a dose as low as 1 mg/kg with a brain concentration of 37 ng/mL (87 nM; 3.0 nM free). At 3 mg/kg, sustained Aβ reduction was observed because the brain level at 6 h (611 nM; 73 nM free) clearly exceeded a cellular IC_{50} value of 0.38 nM. Beagle dogs received 0.08, 0.16, 0.31, and 1.25 mg/kg oral doses of **5**. Plasma samples were taken at 0.5, 1, 2, 4, 8, 25, and 49 h, and cerebrospinal fluid (CSF) was sampled in conscious animals from the lateral ventricle before dosing and at 4, 8, 25, and 49 h. Single oral dose of **5** also produced robust and dose-dependent $Aβ_{42}$ reductions in the CSF. As shown in Figure 3, maximal $Aβ_{42}$ reductions (~80%) were achieved at 1.25 mg/kg, and **5** achieved 50% reduction even at 0.08 mg/kg. Consistent with the high $K_{p,uu}$ in rat and mouse as well as the low P-gp efflux ratio, the CSF-to-unbound plasma concentration ratio in dog was measured to be 0.77 (an average of 0.08, 0.16, and 0.31 mg/kg). Finally, the plasma EC50 and EC80 values to lower CSF $Aβ_{42}$ by 50% and 80% were determined to be 8.9 ng/mL (21 nM) and 44 ng/mL (100 nM), respectively, resulting in a low unbound EC_{50} ($EC_{50,u}$) of 0.69 ng/mL (1.8 nM; Table 2). We were also interested in the potency in dog between **5** and the clinical compounds verubecestat and elenbecestat. As shown in Table 2, compound **5** was found to be more potent than both when considering the $EC_{50,u}$ values.

Figure 2 here

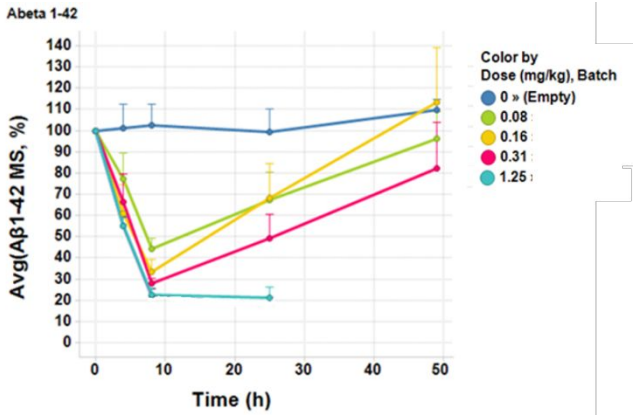


Figure 3. CSF $Aβ_{42}$ reduction in beagle dogs after oral dose of compound **5** as a solution in 20% HPBCD

Table2. Dog EC_{50} values for 5, verubecestat, and elenbecestat

	EC_{50} (nM)	$EC_{50,u}$ (nM)
5	21	1.8
verubecestat	34	11
elenbecestat	22	8.1

Human Dose Projection. Prior to generating human dose projections, PK parameters of **5** were determined in rat, mouse, dog, and monkey (Table 3). Reflecting the stability in hepatocytes, clearance in dog was low (3 mL/min/kg), whereas that in mouse, rat, and monkey was found to be moderate-to-high. Indeed, the low clearance in dog was translated into a high bioavailability of 95%, the others were found to be moderate (24 to 58%). For atabecestat with a thiazine scaffold, the shape of the PK profile was better predicted using the Dedrick approach[15] compared to the allometric scaling approach. Therefore, it would be appropriate to rely on the Dedrick approach for **5**. To predict human dosage, the following assumptions were made: an absorption rate (k_a) of 0.47 (identical to atabecestat) and an oral bioavailability of 54% (average from preclinical species testing), where the target plasma trough levels (C_{trough}) were set to the EC_{50} and EC_{80} values. As a result, a once daily dose of 14 mg with a C_{max} of 70 ng/mL (165 nM, 13.7 nM free) and an AUC of 1403 ng·h/mL (3298 nM·h/mL; 273 nM·h/mL free) was projected to maintain C_{trough} at the EC_{80} (achieving Aβ reduction of 80%), whereas a daily dose of 2.9 mg was required to maintain 50% Aβ reduction. In the multiple ascending doses (MAD) of verubecestat and elenbecestat in healthy adults, once daily doses of 40 and 75 mg were required to reduce Aβ by about 80%, respectively; atabecestat at 30 mg was needed to achieve 80% Aβ reduction in the MAD study,[10] implying that **5** could be used at a reduced human daily dosage relative to the clinical compounds as well as to achieve around 10 mg/day even with approximate maximum Aβ reduction. Having established a high in vivo potency and a low projected human dosage, the development of **5** progressed to in vivo cardiovascular safety and 2-week exploratory toxicology studies.

Table 3. Pharmacokinetic profiles of 5 in Preclinical Species[a]

	mouse	rat	dog	monkey
dose (iv)	0.5	0.5	0.5	0.5
CL (mL/min/kg)	41	27	2.6	23
Vdss (L/kg)	7.5	5.0	2.8	7.1
t1/2 (h)	3.2	2.2	13.7	4.9
dose (po)	2.5	2.5	2.5	2.5
Cmax (ng/mL)	195	148	685	54
Tmax (h)	0.3	0.7	1.8	3.0
AUC (ng·h/mL)	596	566	10,366	429
F (%)	58	38	95	24

[a]Dosed as a solution of **15** in 20% HPBCD.

Cardiovascular Safety. The cardiovascular safety of **5** was evaluated in guinea pig and dog.[14] In anesthetized guinea pigs (n = 7), **5** was administered intravenously at doses of 0.19, 0.38, 0.75, 1.5, 3 and 6 mg/kg over a period of 5 min at 15 min intervals. Compound **5** had no statistically significant effects on QTcB intervals compared to the vehicle, where the no observed adverse effect level (NOAEL) was measured to be more than 6.9 μg/mL (2.4 μM free) with a safety margin of more than 175-fold over the human projected unbound C_{max} sustaining Aβ reduction by 80% at trough level. In anesthetized dogs, increasing intravenous doses of 0.08, 0.16, 0.32, 0.63, 1.25 and 2.5 mg/kg were infused over 5 min at 30 min intervals. Similar to the study with guinea pigs, **5** did not show QTc prolongation and other physiologically relevant effects. Based on the NOAEL of more than 6.9 μg/mL (1.3 μM free), the safety margin was calculated to be more than 95-fold. These results demonstrated the excellent cardiovascular safety of compound **5**.

In Vivo Toxicology. Prior to the 2-week tolerance study, a single ascending dose study was carried out with rat (0, 100, 500, and 2000 mg/kg; n = 5) and dog (0, 25, 50, and 100 mg/kg; n = 2), in which adverse events such as central nervous system (CNS) symptoms and body weight loss were observed at the highest doses for both rats and dogs. Sprague-Dawley rats (n = 10) were given oral doses of 0, 100, 250, and 500 mg/kg/day as a suspension of 0.5% methylcellulose for a period of 2 weeks. With the 500 mg/kg/day dose, reduced body weight and food consumption as well as stomach necrosis were considered toxicologically relevant, and the medium dose of 250 mg/kg/day was considered the NOAEL (AUC = 68 μg·h/mL) with a safety margin of 49-fold over the projected human AUC achieving sustained 80% Aβ reduction. Beagle dogs (n = 4) were given oral doses of 6, 20, and 50 mg/kg/day using the same formulation protocol for a period of 2 weeks. At 50 mg/kg/day, CNS symptoms, such as convulsions, tremors, twitching, vomiting, and salivation, were pronounced; at 20 mg/kg/day, tremors were observed. On the basis of these results, the NOAEL was considered to be 6 mg/kg/day (AUC = 41 μg·h/mL) with a safety margin of 29-fold over the human projected AUC. Importantly, no clinical or histopathological findings derived from liver toxicity were observed in the 2-week tolerance study of **5**, whereas the toxicity study for the same period of atabecestat at the highest dose showed indications of liver toxicity such as alanine aminotransferase elevation and inflammatory cell infiltration of centrilobular hepatocytes. With respect to **5**, the lower liver toxicity as well as the excellent cardiovascular safety profiles supported its advancement to Investigational New Drug enabling studies. Unfortunately, in a 1-month GLP toxicity study with rat, **5** exhibited a rare phospholipidosis (PLD) in the brain at the highest dose, resulting in neuronal cell death. This significant finding suspended further evaluation of compound **5**. A 1,4-oxazine-based BACE1 inhibitor exhibited PLD in the peripheral tissues in a 1-month rat toxicology study, although it was fond to be reversible.[16a] PLD is related to lipophilicity for basic compounds, and cationic amphiphilic compounds with cLogP >3 and pK_a >8 generally cause PLD. Given its acceptable physicochemical profile (LogD = 2.4 and pK_a = 7.3), it was unclear why **5** exhibited PLD.[16b]

CONCLUSIONS

A pK_a lowering strategy based on an oxazine lead **1** led to the discovery of thiazine **5** with increased cellular potency retaining other favorable properties observed in **1**, such as hERG inhibitory activity and P-gp efflux. The improved cellular potency reflected robust Aβ reduction in vivo at low concentrations, leading to a low human projected dose of 14 mg/day to achieve Aβ reduction of 80% at trough level. Compound **5** offered sufficient safety margins in the preclinical cardiovascular safety models. A difference in liver toxicity profile between **5** and atabecestat was noted in the 2-week tolerance study in dog, where **5** showed no significant adverse events related to liver toxicity compared to atabecestat, which led to **5** being considered as a suitable backup candidate for atabecestat. However, further work on **5** was stopped due to unexpected phospholipidosis in the brain during its IND enabling study.

EXPERIMENTAL SECTION

General Chemistry. All commercial reagents and solvents were used as received unless otherwise noted. Flash column chromatography was carried out with an automated purification system using Yamazen or Fuji Silysia prepacked silica gel columns. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Advance 400 and 100 MHz spectrometers, respectively. Analytical LC/MS was performed with a Shimadzu Shim-pack XR-ODS (C18, 2.2 μm, 3.0 × 50 mm, a linear gradient from 10% to 100% B over 3 min and then 100% B for 1 min (A = H2O + 0.1% formic acid, B = MeCN + 0.1% formic acid), flow rate 1.6 mL/min) using a Shimadzu UFLC system equipped with a LCMS-2020 mass spectrometer, LC-20AD binary gradient module, SPD-M20A photodiode array detector (detection at 254

nm), and SIL-20AC sample manager. The purity of all compounds used in the bioassays was determined by this method to be >95%. High resolution mass spectra were recorded with a Thermo Fisher Scientific LTQ Orbitrap using electrospray positive ionization.

***N*-(3-((4*R*,5*R*,6*R*)-2-Amino-5-fluoro-4,6-dimethyl-5,6-dihydro-4*H*-1,3-thiazin-4-yl)-4-fluorophenyl)-5-(fluoromethoxy)pyrazine-2-carboxamide (5).** To a solution of **11** (3.40 g, 13.3 mmol) in TFA (32.0 mL) was added sulfuric acid (8.00 mL) dropwise at –20 °C and then fuming HNO_3 (0.593 mL, 13.3 mmol). After stirring for 1 h at –20 °C , the mixture was poured into aqueous K_2CO_3 solution at 0 °C. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated to give the crude compound (4.43 g) as a tan amorphous substance. To a solution of this crude compound (4.43 g) in toluene (40.0 mL) and H_2O (40.0 mL) were added Fe (6.24 g, 112 mmol) and NH_4Cl (8.97 g, 168 mmol). The reaction mixture was heated to 80 °C for 1.5 h and then cooled to room temperature. The mixture was diluted with aqueous K_2CO_3 solution and filtered through Celite. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated to give crude compound (3.36 g) as a tan solid. To a solution of this crude compound (332 mg), 5-(fluoromethoxy)pyrazine-2-carboxylic acid (211 mg, 1.22 mmol), and HCl in water (2 M in water; 612 µL, 1.22 mmol) in MeOH was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (258 mg, 1.35 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h and then quenched with aqueous $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated. The crude compound was crystallized with acetone/H_2O (1:3) to give **5** (484 mg, 91% over 3 steps) as a white solid. The stereochemistry was confirmed by ^{19}F-^{1}H HOESY and a cocrystal structure of **5** bound to BACE(Figure S5; See Supporting Information). ^{1}H NMR (400 MHz, CDCl3) δ 9.47 (1H, br s), 9.08 (1H, d, *J* = 1.1 Hz), 8.30 (1H, d, *J* = 1.1 Hz), 7.99 (1H, ddd, *J* = 8.9, 4.0, 2.8 Hz), 7.29 (1H, dd, *J* = 6.8, 2.8 Hz), 7.09 (1H, dd, *J* = 11.5, 8.9 Hz), 6.15 (2H, ddd, *J* = 51.1, 4.5, 2.0 Hz), 5.12 (1H, dd, *J* = 47.6, 1.4 Hz), 3.16-3.03 (1H, m), 1.77 (3H, s), 1.34 (3H, d, *J* = 6.9 Hz). ^{13}C NMR (100 MHz, CDCl3) δ 160.4, 159.3 (d, *J* = 2.5 Hz), 155.6 (d, *J* = 243.8 Hz), 152.0, 141.8, 139.6, 133.9 (d, *J* = 2.4 Hz), 133.2, 131.9 (dd, *J* = 14.4, 6.4 Hz), 120.7 (d, *J* = 8.6 Hz), 120.5 (br-d, *J* = 3.1 Hz), 117.2 (d, *J* = 25.7 Hz), 95.8 (d, *J* = 223.6 Hz), 86.5 (dd, *J* = 185.7, 7.3 Hz), 62.04 (dd, *J* = 21.1, 5.2 Hz), 36.0 (d, *J* = 20.8 Hz), 26.5 (t, *J* = 4.0 Hz), 17.7 (d, *J* = 2.4 Hz). HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $[C_{18}H_{19}O_2N_5F_3S]^+$ 426.1206, found 426.1201.

***tert*-Butyl ((2*R*,3*S*)-3-fluoro-2-(2-fluorophenyl)pent-4-en-2-yl)carbamate (7).** To a solution of **6** (18.8 g, 54.8 mmol) in DCM (190 mL) was added a solution of DIBAL (1.02 M, in hexane; 177 mL, 181 mmol) at –78 °C. The resulting solution was stirred at –78 °C for 2.5 h. The reaction mixture was poured into a aqueous Rochelle's solution and extracted with DCM. The combined organic layers were washed with water and brine, dried over $Na2SO_4$, and concentrated to give the crude compound (16.5 g) as a yellow oil. To a solution of methyltriphenylphosphonium bromide (48.9 g, 137 mmol) in toluene (250 mL) was added KO*t*-Bu (1.0 M, in THF; 126 mL, 126 mmol) at room temperature. The mixture was stirred at room temperature for 1 h, and then a solution of the crude compound in toluene (50.0 mL) was added at 0 °C. The resulting mixture was stirred at room temperature overnight, and then quenched with aqueous NH_4Cl solution. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated. The crude compound was purified by silica gel column chromatography eluting with 5–20% EtOAc in hexane to provide compound **7** (6.35 g, 21.4 mmol, 39% in 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (1H, t, J = 8.0 Hz), 7.28–7.24 (1H, m), 7.12 (1H, td, *J* = 7.8, 1.4 Hz), 7.02 (1H, dd, *J* = 12.7, 8.2 Hz), 5.74-5.62 (1H, m), 5.47–5.30 (3H, m), 5.17 (1H, br s), 1.86 (3H, s), 1.38 (9H, s).

***N*-(((2*R*,3*S*)-3-Fluoro-2-(2-fluorophenyl)pent-4-en-2-yl)carbamothioyl)benzamide (8).** To a solution of **7** (6.35 g, 21.4 mmol) in DCM (40.0 mL) was added 4 mol/L of HCl in dioxane (26.7 mL, 107 mmol) at room temperature, and the mixture was stirred for 1 h. The mixture was poured into aqueous $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated to give the crude compound (4.74 g) as a tan liquid. To a solution of this crude compound in DCM (40 mL) was added benzoyl isothiocyanate (3.16 mL, 23.5 mmol) at 0 °C. The resulting mixture was warmed to room temperature and stirred overnight. The reaction solution was concentrated and the residue was purified by silica gel column chromatography using 5–20% EtOAc in hexane to provide compound **8** (7.29 g, 20.2 mmol, 95% in 2 steps). ^{1}H NMR (400 MHz, CDCl3) δ 11.51 (1H, s), 8.83 (1H, s), 7.85 (2H, d, J = 7.4 Hz), 7.63 (1H, t, *J* = 7.4 Hz), 7.52 (2H, t, *J* = 7.7 Hz), 7.44 (1H, t, *J* = 8.0 Hz), 7.33–7.27 (1H, m), 7.16 (1H, td, *J* = 7.8, 1.0 Hz), 7.04 (1H, ddd, *J* = 12.5, 8.2, 1.0 Hz), 5.95-5.83 (1H, m), 5.69 (1H, dd, *J* = 46.4, 6.1 Hz), 5.66–5.62 (1H, m), 5.49 (1H, d, *J* = 10.9 Hz), 2.13 (3H, s). MS-ESI (*m*/*z*): 361 $[M + H]^+$.

***N*-((4*R*,5*R*,6*S*)-5-Fluoro-4-(2-fluorophenyl)-6-(iodomethyl)-4-methyl-5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide (9).** To a solution of **8** (7.29 g, 20.2 mmol) in MeCN (80 mL) was added iodine (10.3 g, 40.5 mmol) at 0 °C. After stirring for 1 h, the mixture was diluted with aqueous $NaHCO_3$ solution and aqueous $Na_2S_2O_3$ solution. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated. The crude compound was purified by silica gel column chromatography eluting with 5–20% EtOAc in hexane to provide compound **9** (8.04 g, 16.5 mmol, 82%). ^{1}H NMR (400 MHz, CDCl3) δ 11.67 (1H, br s), 8.21 (2H, d, *J* = 7.4 Hz), 7.53 (1H, t, J = 7.3 Hz), 7.45 (2H, t, *J* = 7.5 Hz), 7.41-7.36 (1H, m), 7.34 (1H, t, *J* = 7.8 Hz), 7.20 (1H, t, *J* = 8.0 Hz), 7.15 (1H,

dd, *J* = 12.2, 8.2 Hz), 5.70 (1H, d, *J* = 47.7 Hz), 3.53-3.48 (1H, m), 3.27-3.15 (2H, m), 1.90 (3H, s). MS-ESI (*m*/*z*): 487 [M + H]$^+$.

***N*-((4*R*,5*R*,6*R*)-5-Fluoro-4-(2-fluorophenyl)-4,6-dimethyl-5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide (10).** To a solution of **9** (8.04 g, 16.5 mmol) in toluene (80 mL) were added Bu_3SnH (6.62 mL, 24.8 mmol) and AIBN (0.136 g, 0.827 mmol). The solution was heated to 80 °C for 2 h and then cooled to room temperature. The mixture was concentrated, and the residue was purified by silica gel column chromatography eluting with 5–20% EtOAc in hexane to provide compound **10** (5.35 g, 14.8 mmol, 90%). ^{1}H NMR (400MHz, $CDCl_3$) δ 12.07 (1H, br s), 8.23 (2H, d, *J* = 7.5 Hz), 7.51 (1H, t, *J* = 7.3 Hz), 7.44 (2H, t, *J* = 7.5 Hz), 7.40-7.34 (2H, m), 7.20 (1H, t, *J* = 7.6 Hz), 7.11 (1H, dd, *J* = 12.2, 8.2 Hz), 5.26 (1H, d, *J* = 46.9 Hz), 3.13 (1H, dq, *J* = 31.3, 6.9 Hz), 1.87 (3H, s), 1.41 (3H, d, *J* = 6.9 Hz). MS-ESI (*m*/*z*): 361 [M + H]$^+$.

(4*R*,5*R*,6*R*)-5-Fluoro-4-(2-fluorophenyl)-4,6-dimethyl-5,6-dihydro-4*H*-1,3-thiazin-2-amine (11). To a solution of **10** (5.35 g, 14.8 mmol) in EtOH (50.0 mL) was added hydrazine hydrate (3.60 mL, 74.2 mmol) at room temperature. After stirring overnight, the reaction mixture was concentrated. The resulting residue was purified by silica gel column chromatography eluting with 20–70% EtOAc in hexane to provide compound **11** (3.45 g, 13.5 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.24 (2H, m), 7.12 (1H, t, *J* = 7.5 Hz), 7.03 (1H, dd, *J* = 12.0, 8.3 Hz), 5.09 (1H, d, *J* = 47.7 Hz), 4.55 (2H, br s), 3.02 (1H, dq, *J* = 31.1, 6.8 Hz), 1.74 (3H, s), 1.32 (3H, d, *J* = 6.8 Hz). MS-ESI (*m*/*z*): 257 [M + H]$^+$.

Biochemical BACE1 Assay. The biochemical BACE1 IC_{50} values were determined by an HTRF assay using an APP derived peptide as described previously.[11]

Cellular Aβ Assay. The cellular Aβ IC_{50} values were determined by measuring Aβ40 using a HTRF assay in SH-SY5Y cells expressing human APP as described previously.[11]

In Vitro ADMET Assays. Metabolic stability in microsomes, P-gp efflux ratio, solubility, protein binding, brain tissue binding, CYP IC_{50}, and hERG inhibitory activity were determined according to procedures described previously.[11]

In Vivo Experiments. In vivo experimental studies were performed at Janssen and Shionogi. All the procedures were in accordance with regulations and established guidelines and were approved by the local ethical committees of both companies: the Ethical Committee on Laboratory Animal Testing (ECD, Janssen Beerse) and the Shionogi Animal Care and Use Committee.

In Vivo Pharmacokinetic Study. The test compound was dissolved in 20% HPBCD, and the detailed method was described previously.[11]

Mouse PK/PD Study. The test compound was dissolved in 20% HPBCD and was orally administered to male Crl:CD1 (ICR) mouse (6 to 8 weeks old; n = 4) at 1 and 3 mg/kg doses. The total Aβ levels were measured using an ELISA method, and the details of which were described previously.[11a,b]

Dog PK/PD Study. The effect of the compounds on the Aβ profile in CSF of Beagle dogs was tested in combination with PK follow up. The lowering effect was described as EC_{50} and EC_{80} values, which are defined as the plasma level of a tested compound required for 50% and 90% lowering of Aβ in CSF. These values were determined after testing of the compound for dose response, using the statistical methods. The detailed method was described previously.[14]

In Vivo Toxicology Study in Rat. A single-dose oral toxicity study was conducted in male Sprague-Dawley rats (*n* = 5 per group). The test compound was administered at 0, 100, 500, or 2,000 mg/kg (dose volume = 10 mL/kg) and was formulated as an aqueous suspension containing 0.5% (w/v) methylcellulose. A 2-week repeated-dose oral toxicity study with the test compound was conducted with male Sprague-Dawley rats (*n* = 5 per main group) at 0, 100, 250, and 500 mg/kg/day as an aqueous suspension in 0.5% (w/v) methylcellulose. For toxicokinetic purposes, an additional 3 rats per test compound-treated group were included. Toxicokinetic Parameters of **5** in Rat and Dog are given in Table S1-S5 (See Supporting Information).

In Vivo Toxicology Study in Dog. A single-dose oral toxicity study was conducted with female beagle dogs (*n* = 2 per group). A dose of 100 mg/kg was administered as a non-clear aqueous solution containing 25% w/v HPBCD (HCl to pH 2; dose volume = 5 mL/kg) or as a suspension in 0.5% (w/v) methylcellulose aq. (dose volume = 2 mL/kg). A 2-week repeated-dose oral toxicity study was conducted with male and female beagle dogs (*n* = 2 per group) at 6, 20, and 60 mg/kg/day as a suspension in 0.5% (w/v) methylcellulose aq. For toxicokinetic purposes, the same dogs per test compound-treated group were used.

ASSOCIATED CONTENT

Supporting Information. Toxicokinetic parameters of **5** in rat and dog, experimental procedures for in vivo cardiovascular safety study, human dose and PK projections, ^{1}H NMR, ^{13}C NMR, ^{19}F-^{1}H HOESY data for **5**, and crystallographic data. (PDF)

SMILES strings, BACE1 IC_{50}, cellular $Aβ_{40}$ IC_{50} (CSV)

Accession Codes

The PDB accession code for the X-ray structure of **5** bound to human BACE1 is 6JT3.

AUTHOR INFORMATION

Corresponding Author

*For K.K: Phone, +81-6-6331-6190; Fax: +81-6-6332-6385. E-mail: ken-ichi.kusakabe@shionogi.co.jp

ORCID

Harrie. J. M. Gijsen: 0000-0001-8020-4848

Ken-ichi Kusakabe: 0000-0001-9055-8267

Present Addresses

[#]API Process Development Department (Biotechnology),

Pharmaceutical Technology Division, Chugai Pharmaceutical Co., Ltd, 5-1, Ukima 5-chome, Kita-ku, Tokyo, 115-8543 Japan.
◊Sosei Heptares, Steinmetz Building, Granta Park, Great Abington, Cambridge CB21 6DG, United Kingdom.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

We thank Maki Hattori, Masanori Nakae, and Maito Douma for the biological assays, Yumiko Takagi for the elemental analysis, Yuki Aoe for the HRMS analysis, and Yukiko Kan for the NMR analysis. We gratefully thank the members of the Janssen-Shionogi research collaboration for the support of this program. We are grateful to Judy Noguchi for proofreading the manuscript.

ABBREVIATIONS

Alzheimer's disease, AD; amyloid-β, Aβ; APP, amyloid precursor protein; BACE1, the β-site amyloid precursor protein cleaving enzyme 1; Boc, *tert*-butoxycarbonyl; CNS, central nervous system; CSF, cerebrospinal fluid; DIBAL, diisobutylaluminium hydride; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydro-chloride; HPBCD, hydroxypropyl-β-cyclodextrin; MAD, multiple ascending dose; NOAEL, no observed adverse effect level; PD, pharmacodynamic; PK, pharmacokinetic; PLD, phospholipidosis.

REFERENCES

1. Prince, M.; Comas-Herrera, A.; Knapp, M.; Guerchet, M.; Karagiannidou, M. World Alzheimer Report 2016. https://www.alz.co.uk/research/WorldAlzheimerReport2016.pdf (accessed February 20, 2018).
2. Selkoe, D. J.; Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* **2016**, *8*, 595–608.
3. Jonsson, T.; Atwal, J. K.; Steinberg, S.; Snaedal, J.; Jonsson, P. V.; Bjornsson, S.; Stefansson, H.; Sulem, P.; Gudbjartsson, D.; Maloney, J.; Hoyte, K.; Gustafson, A.; Liu, Y.; Lu, Y.; Bhangale, T.; Graham, R. R.; Huttenlocher, J.; Bjornsdottir, G.; Andreassen, O. A.; Jönsson, E. G.; Palotie, A.; Behrens, T. W.; Magnusson, O. T.; Kong, A.; Thorsteinsdottir, U.; Watts, R. J.; Stefansson, K. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* **2012**, *488*, 96–99.
4. Yan, R.; Vassar, R. Targeting the β secretase BACE1 for Alzheimer's disease therapy. *Lancet Neurol.* **2014**, *13*, 319–329.
5. (a) Oehlrich, D.; Prokopcova, H.; Gijsen, H. J. M. The evolution of amidine-based brain penetrant BACE1 inhibitors. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2033–2045. (b) Hall, A.; Gijsen, H. J. M. Targeting β-secretase (BACE) for the treatment of Alzheimer's disease. In *Comprehensive Medicinal Chemistry III*, 3rd ed.; Chackalamannil, S., Rotella, D. P., Ward, S. E., Eds.; Elsevier: Oxford, 2017; vol. 7, pp 326–383. (c) Hsiao, C.-C.; Rombouts, F.; Gijsen, H. J. M. New evolutions in the BACE1 inhibitor field from 2014 to 2018. *Bioorg. Med. Chem. Lett.* DOI: 10.1016/j.bmcl.2018.12.049.
6. (a) Scott, J. D.; Li, S. W.; Brunskill, A. P. J.; Chen, X.; Cox, K.; Cumming, J. N.; Forman, M.; Gilbert, E. J.; Hodgson, R. A.; Hyde, L. A.; Jiang, Q.; Iserloh, U.; Kazakevich, I.; Kuvelkar, R.; Mei, H.; Meredith, J.; Misiaszek, J.; Orth, P.; Rossiter, L. M.; Slater, M.; Stone, J.; Strickland, C. O.; Voigt, J. H.; Wang, G.; Wang, H.; Wu, Y.; Greenlee, W. J.; Parker, E. M.; Kennedy, M. E.; Stamford, A. W. Discovery of the 3-imino-1, 2,4-thiadiazinane 1,1-dioxide derivative verubecestat (MK-8931)–A β-site amyloid precursor protein cleaving enzyme 1 inhibitor for the treatment of Alzheimer's disease *J. Med. Chem.* **2016**, *59*, 10435–10450. (b) Egan, M. F.; Kost, J.; Tariot, P. N.; Aisen, P. S.; Cummings, J. L.; Vellas, B.; Sur, C.; Mukai, Y.; Voss, T.; Furtek, C.; Mahoney, E.; Mozley, L. H.; Vandenberghe, R.; Mo, Y.; Michelson, D. Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. *N. Engl. J. Med.* **2018**, *378*, 1691–1703. (c) Merck & Co., Inc. https://investors.merck.com/news/press-release-details/2018/Merck-Announces-Discontinuation-of-APECS-Study-Evaluating-Verubecestat-MK-8931-for-the-Treatment-of-People-with-Prodromal-Alzheimers-Disease/default.aspx (accessed January 7, 2019)
7. (a) Eketjaell, S.; Janson, J.; Kaspersson, K.; Bogstedt, A.; Jeppsson, F.; Faelting, J.; Haeberlein, S. B.; Kugler, A. R.; Alexander, R. C.; Cebers, G.; Ho, P. AZD3293: A novel, orally active BACE1 inhibitor with high potency and permeability and markedly slow off-rate kinetics. *J. Alzheimers Dis.* **2016**, *50*, 1109–1123. (b) Alzforum. https://www.alzforum.org/therapeutics/azd3293 (accessed December 23, 2018)
8. (a) Alzforum. http://www.alzforum.org/therapeutics/elenbecestat (accessed July 24, 2017) (b) 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). WO 2009091016 A1, July 23, 2009.
9. (a) Neumann, U.; Ufer, M.; Jacobson, L. H.; Rouzade-Dominguez, M.-L.; Huledal, G.; Kolly, C.; Lueoend, R. M.; Machauer, R.; Veenstra, S. J.; Hurth, K.; Rueeger, H.; Tintelnot-Blomley, M.; Staufenbiel, M.; Shimshek, D. R.; Perrot, L.; Frieauff, W.; Dubost, V.; Schiller, H.; Vogg, B.; Beltz, K.; Avrameas, A.; Kretz, S.; Pezous, N.; Rondeau, J.; Beckmann, N.; Hartmann, A.; Vormfelde, S.; David, O. J.; Galli, B.; Ramos, R.; Graf, A.; Lopez Lopez, C. The BACE-1 inhibitor CNP520 for prevention trials in Alzheimer's disease. *EMBO Mol. Med.* **2018**, *10*, e9316. (b) Novartis AG. https://www.globenewswire.com/news-release/2019/07/11/1881745/0/en/Novartis-Amgen-and-Banner-Alzheimer-s-Institute-discontinue-clinical-program-with-BACE-inhibitor-CNP520-for-Alzheimer-s-prevention.html (accessed September 1, 2019)
10. (a) Timmers, M.; Van Broeck, B.; Ramael, S.; Slemmon, J.; De Waepenaert, K.; Russu, A.; Bogert, J.; Stieltjes, H.; Shaw, L. M.; Engelborghs, S.; Moechars, D.; Mercken, M.; Liu, E.; Sinha, V.; Kemp, J.; Van Nueten, L.; Tritsmans, L.; Streffer, J. R. Profiling the dynamics of CSF and plasma Aβ reduction after treatment with JNJ-54861911, a potent oral BACE inhibitor. *Alzheimers Dement (N Y)* **2016**, *2*, 201–212. (b) The Janssen Pharmaceutical Companies of Johnson & Johnson. https://www.janssen.com/ja/node/45771 (accessed December 23, 2018)
11. (a) Nakahara, K.; Fuchino, K; Komano, K; Asada, N; Tadano, G; Hasegawa, T; Yamamoto, T; Sako, Y; Ogawa, M; Unemura, C; Hosono, M; Ito, H; Sakaguchi, G; Ando, S; Ohnishi, S; Kido, Y; Fukushima, T; Dhuyvetter, D; Borghys, H; Gijsen, H. J. M.; Yamano, Y; Iso, Y; Kusakabe, K.-I. Discovery of potent and centrally active 6-substituted 5-fluoro-1,3-dihydro-oxazine β-secretase (BACE1) inhibitors via active conformation stabilization. *J. Med. Chem.* **2018**, *61*, 5525–5546. (b) Fuchino, K.; Mitsuoka, Y.; Masui, M.; Kurose, N.; Yoshida, S.; Komano, K.; Yamamoto, T.; Ogawa, M.; Unemura, C.; Hosono, M.; Ito. H.; Sakaguchi, G.; Ando, S.; Ohnishi, S.; Kido, Y.; Fukushima, T.; Miyajima, H.;

Hiroyama, S.; Koyabu, K.; Dhuyvetter, D.; Borghys, H.; Gijsen, H. J.M.; Yamano, Y.; Iso, Y.; Kusakabe, K.-I. Rational design of novel 1,3-oxazine based β-secretase (BACE1) inhibitors: Incorporation of a double bond to reduce P-gp efflux leading to robust Aβ reduction in the brain. *J. Med. Chem.* **2018**, *61*, 5122–5137.

12. (a) Stepan, Antonia F.; Walker, Daniel P.; Bauman, Jonathan; Price, David A.; Baillie, Thomas A.; Kalgutkar, Amit S.; Aleo, Michael D. Structural alert/reactive metabolite concept as applied in medicinal chemistry to mitigate the risk of idiosyncratic drug toxicity: A perspective based on the critical examination of trends in the top 200 drugs marketed in the United States. *Chem. Res. Toxicol.* **2011**, *24*, 1345–1410. (b) Thompson, R. A.; Isin, E. M.; Li, Y.; Weidolf, L.; Page, K.; Wilson, I.; Swallow, S.; Middleton, B.; Stahl, S.; Foster, A. J.; Dolgos, H.; Weaver, R.; Kenna, J. G. In vitro approach to assess the potential for risk of idiosyncratic adverse reactions caused by candidate drugs. *Chem. Res. Toxicol.* **2012**, *25*, 1616–1632.
13. Representative thiazine-based BACE1 inhibitors: (a) Kobayashi, N.; Ueda, K.; Itoh, N.; Suzuki, S.; Sakaguchi, G.; Kato, A.; Yukimasa, A.; Hori, A.; Kooriyama, Y.; Haraguchi, H.; Yasui, K.; Kanda, Y. Preparation of 2-Amino-4-phenyl-4,5-dihydro-5*H*-1,3-thiazine Derivatives and Related Compounds for Treatment of Alzheimer's Disease. WO 2008133273 A1, November 6, 2008. (b) 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.
14. Borghys, H.; Jacobs, T.; Van Broeck, B.; Dillen, L.; Dhuyvetter, D.; Gijsen, H.; Mercken, M. Comparison of two different methods for measurement of amyloid-β peptides in cerebrospinal fluid after BACE1inhibition in a dog model. *J. Alzheimers Dis.* **2014**, *38*, 39–48.
15. Dedrick R. L. *J. Pharmacokinet. Biopharm.* **1973**, *1*, 435.
16. (a) Gijsen, H. J. M.; Alonso de Diego, S. A.; De Cleyn, M.; Garcia-Molina, A.; Macdonald, G. J.; Martinez-Lamenca, C.; Oehlrich, D.; Prokopcova, H.; Rombouts, F. J. R.; Surkyn, M.; Trabanco, A. A.; Van Brandt, S.; Van den Bossche, D.; Van Gool, M.; Austin, N.; Borghys, H.; Dhuyvetter, D.; Moechars, D. Optimization of 1,4-oxazine β-secretase 1 (BACE1) inhibitors toward a clinical candidate. (b) 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.

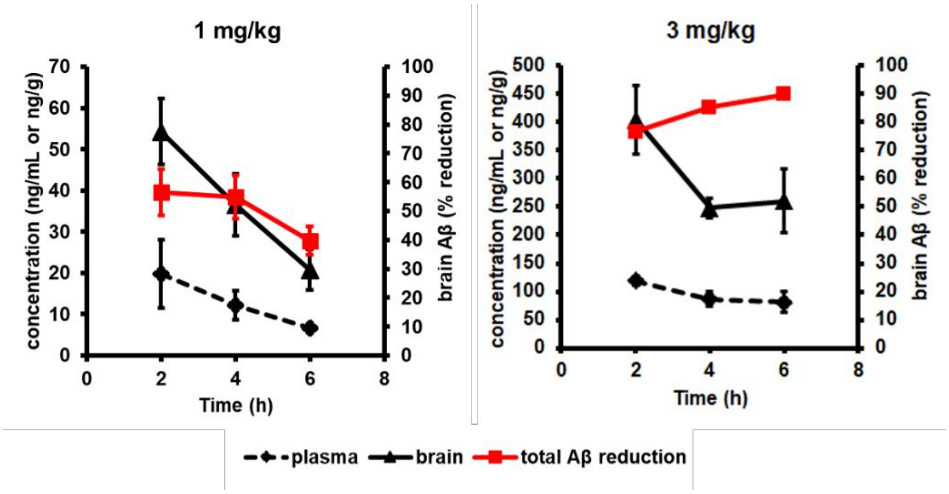
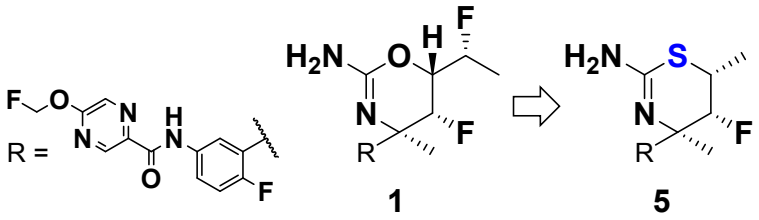


Figure 2. Time course effects of **5** on total brain Aβ reduction (vs vehicle control), plasma, and brain concentrations in male Crl:CD1 (ICR) mice (6 to 8 weeks old, $n = 4$) after 1 and 3 mg/kg oral doses as a solution of 20% hydroxypropyl-β-cyclodextrin (HPBCD).[11a,b] Total Ab reductions (% reduction; red) and brain concentrations (ng/g; black) are shown by solid lines, and plasma concentrations (ng/mL; black) is shown by dashed lines.

Table of Contents graphic



	1	**5**
	pK_a = 7.2	pK_a = 7.3
BACE1 IC_{50}	12 nM	5.0 nM
Cell Aβ IC_{50}	1.1 nM	0.38 nM
Dog Aβ EC_{50}	32 ng/mL	8.9 ng/mL

Projected Human Dose = 14 mg/day
(80% Aβ reduction)