# Journal of Medicinal Chemistry

# Article

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# Rational Design of Novel 1,3-Oxazine Based #-Secretase (BACE1) Inhibitors: Incorporation of a Double Bond to Reduce P-gp Efflux Leading to Robust A# Reduction in the Brain

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# 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

Kouki Fuchino,<sup>†,∞</sup> Yasunori Mitsuoka,<sup>†,∞</sup> Moriyasu Masui,<sup>†,∞</sup> Noriyuki Kurose,<sup>†</sup> Shuhei Yoshida,<sup>†</sup> Kazuo Komano,<sup>†</sup> Takahiko Yamamoto,<sup>†</sup> Masayoshi Ogawa,<sup>†</sup> Chie Unemura,<sup>†</sup> Motoko Hosono,<sup>†</sup> Hisanori Ito,<sup>†</sup> Gaku Sakaguchi,<sup>†</sup> Shigeru Ando,<sup>‡</sup> Shuichi Ohnishi,<sup>‡</sup> Yasuto Kido,<sup>‡</sup> Tamio Fukushima,<sup>‡</sup> Hirofumi Miyajima,<sup>‡</sup> Shuichi Hiroyama,<sup>‡</sup> Kiyotaka Koyabu,<sup>‡</sup> Deborah Dhuyvetter,<sup>¶</sup> Herman Borghys,<sup>¶</sup> Harrie J. M. Gijsen,<sup>§</sup> Yoshinori Yamano,<sup>†</sup> Yasuyoshi Iso,<sup>†</sup> and Ken-ichi Kusakabe<sup>\*,†</sup>

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# ABSTRACT

Accumulation of A $\beta$  peptides is a hallmark of Alzheimer's disease (AD) and is considered a causal factor in the pathogenesis of AD.  $\beta$ -Secretase (BACE1) is a key enzyme responsible for producing A $\beta$  peptides, and thus agents that inhibit BACE1 should be beneficial for disease-modifying treatment of AD. Here we describe the discovery and optimization of novel oxazine-based BACE1 inhibitors by lowering amidine basicity with the incorporation of a double bond to improve brain penetration. Starting from a 1,3-dihydo-oxazine lead **6** identified by a hit-to-lead SAR following HTS, we adopted a p $K_a$  lowering strategy to reduce the P-gp efflux and the high hERG potential leading to the discovery of **15** that produced significant A $\beta$  reduction with long duration in pharmacodynamic models and exhibited wide safety margins in cardiovascular safety models. This compound improved the brain-to-plasma ratio relative to **6** by reducing P-gp recognition, which was demonstrated by a P-gp knockout mouse model.

# **INTRODUCTION**

Alzheimer's disease (AD) is the most common type of dementia. The histopathological hallmarks of AD are neurofibrillary tangles, aggregated and hyper-phosphorylated tau proteins, and amyloid plaques composed of amyloid  $\beta$  (A $\beta$ ) peptides of 38–43 amino acids. Patients with AD die within 3–9 years after diagnosis. In addition to shortening of life expectancy, AD has a significant impact on quality of life both for the patients and their family caregivers. In 2015, over 46 million people were living with dementia worldwide, with AD being the most common form, and the number is estimated to increase to 132 million by 2050.<sup>1</sup> Dementia imposes an enormous economic burden with the total estimated worldwide cost of 818 billion dollars, and the estimated cost for 2018 to be a trillion dollars.<sup>1</sup> In spite of these serious issues, the standard

therapy of Donepezil and Memantine only temporarily relieves disease symptoms. The development of disease-modifying drugs has thus far been unsuccessful.

Strategies targeting inhibition of A $\beta$  peptide production should be important for diseasemodification of AD.<sup>2,3</sup> The  $\beta$ -site amyloid precursor protein cleaving enzyme 1 (BACE1), also known as  $\beta$ -secretase or memapsin2, is an aspartic protease and the rate-limiting enzyme in the A $\beta$  peptide production.<sup>4</sup> First, the amyloid precursor protein (APP) is cleaved by BACE1 to generate the N-terminus of sAPP $\beta$  and the membrane-bound C-terminus of C99. Second, C99 is cleaved by  $\gamma$ -secretase to generate the C-terminus of the A $\beta$  peptides including various isoforms of 38–43 amino acid length, of which the A $\beta$ 42 consisting of 42 amino acids is the most toxic form as it readily aggregates to generate toxic oligomers,<sup>5</sup> which ultimately result in the deposition of amyloid plaques. Although  $\gamma$ -secretase is also considered to be a promising target for reducing A $\beta$  production, its concomitant inhibition of Notch processing has hampered the development of  $\gamma$ -secretase inhibitors for clinical use.<sup>6</sup> BACE1 knockout mice are viable and exhibit a moderate phenotype of morphologic and functional deficit,<sup>3</sup> while deletion of the PSEN-1 gene, the catalytic site of  $\gamma$ -secretase, is embryonically lethal, making BACE1 a more attractive target for A $\beta$ -lowering therapeutics.<sup>6</sup>

Mutations found in APP also provide genetic evidence for the importance of BACE1 in AD. For example, the Swedish mutation (K670N and M671L) is found near the BACE1 cleavage site and makes APP more susceptible to cleavage by BACE1, which ultimately causes familial AD (FAD).<sup>3</sup> In contrast, the A673T mutation is less effectively cleaved by BACE1 and results in an approximately 40% reduction in A $\beta$  peptide formation leading to a protective effect against AD.<sup>3,7</sup> Such evidence partly demonstrates the clinical proof of concept by inhibition of BACE1.

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Significant efforts have been directed at exploring orally available and brain penetrant BACE1 inhibitors since the identification of BACE1 in 1999.<sup>8,9</sup> Initial BACE1 inhibitors were designed based on the transition state or other aspartic proteases such as HIV protease and renin. However, these efforts failed to identify brain penetrant inhibitors with significant in vivo efficacy. Their high molecular weight, polar surface area, and rotatable bond number resulted in compounds with low permeability and high P-gp efflux, which ultimately limited CNS exposure. Identifying small molecules and non-peptidomimetic BACE1 inhibitors was considered to be very challenging due to the large size of the catalytic site in BACE1.<sup>8</sup> The first examples of potent and non-peptide inhibitors with amidine-based scaffolds were reported by scientists at Schering-Plough and Wyeth.<sup>8,10</sup> Since their discovery, many pharmaceutical companies pursued analogs. Although early amidine-based inhibitors retaining high P-gp efflux had low potency in vivo, the challenge was addressed successfully by utilizing structure-based design and controlling physicochemical properties, leading to compounds with robust A $\beta$  reduction in preclinical animal models.<sup>8</sup> Finally, minimizing the risks associated with the high basicity and lipophilicity observed with these analogs, such as high hERG activity and CYP2D6 inhibition, culminated in the discovery of clinical BACE1 inhibitors including 1 (LY-2811376),<sup>11</sup> LY-2886721,<sup>12</sup> 2 (MK-8931, verubecestat),<sup>13</sup> **3** (E-2609, elenbecestat),<sup>14</sup> RG-7129,<sup>15</sup> lanabecestat (AZD-3293),<sup>16a</sup> CNP520,<sup>16b</sup> and JNJ-54861911.<sup>17</sup> Unfortunately, phase I/II studies of several of these compounds were discontinued, most likely due to off-target toxicity.<sup>18</sup> Therefore, identifying BACE1 inhibitors in diverse structural classes was considered to be worthwhile.





In 2003, our early research started with a high-throughput screening (HTS) campaign utilizing a cellular Aß production assay, which successfully identified a thiazine-based hit compound 4 without cellular toxicity (Chart 2). Our early efforts revealed that incorporation of amide groups on the phenyl ring in 4 significantly improved potency leading to the discovery of  $5^{19}$ Furthermore, replacement of the sulfur in 5 with an oxygen atom followed by an amide screen led to  $\mathbf{6}$ , which exhibited comparable cellular potency, while reduced activity was observed in the biochemical assay relative to 5. In parallel with our efforts, Roche also identified the same hit compound of 4 in their HTS campaign.<sup>20</sup> Their efforts utilizing fluorine scans by reducing basicity  $(pK_a)$  on amidine moieties culminated in the CF<sub>3</sub>-substitued dihydro-oxazine 7 with in vivo efficacy (Chart 3).<sup>21</sup> In spite of the excellent profile described for 7, the Roche clinical compound RG-7129, an analog of 7, was terminated in Phase I.<sup>22</sup>

Chart 2. HTS Hit 4 and Initial Leads 5 and 6



BACE1 IC<sub>50</sub>: 73 µM Cell Aβ IC<sub>50</sub>: 2.6 μM



BACE1 IC50: 6.7 nM

Cell Aβ IC<sub>50</sub>: 0.84 nM

O BACE1 IC<sub>50</sub>:



Controlling p $K_a$  is a well-known approach for reducing P-gp efflux as well as mitigating hERG inhibitory activity,<sup>23</sup> particularly in exploring BACE1 inhibitors as with compounds **7**, **8**, and **9** (Chart 3).<sup>9h,21,24</sup> For instance, to lower the p $K_a$  of amidine or amine moieties that interact with the catalytic aspartates in BACE1, incorporation of electron withdrawing groups such as fluorine, oxygen, carbonyl, and sulfone has been successfully applied.<sup>13,20,21,24</sup> Our aim in this work was to improve the P-gp and the hERG liabilities observed with **6** by lowering the p $K_a$ . We describe an alternative approach to reducing the p $K_a$  of the dihydro-oxazine **6** by incorporating a double bond, which successfully lowered the p $K_a$  by 2 log units, leading to oxazine **11** with reduced P-gp efflux. Further modification by fine-tuning the p $K_a$  led to the discovery of compound **15** that showed robust A $\beta$  reduction in mouse and dog, as well as good cardiovascular safety margins in guinea pig and dog over the efficacy level (EC<sub>50</sub>) in dog.

Chart 3. BACE1 Inhibitors 7, 8, and 9 Identified Utilizing a pK<sub>a</sub>-Lowering Strategy



# **RESULTS AND DISCUSSION**

**Design of Novel 1,3-Oxazine BACE1 Inhibitors by Incorporating a Double Bond.** The initial lead **6** displayed moderate to good A $\beta$  inhibitory activity in cells (IC<sub>50</sub> = 3.7 nM); however, it exhibited a high P-gp efflux ratio of 36 in MDCK cells resulting in a low total brain-to-plasma ratio (*B*/*P* or *K*<sub>p</sub>) of 0.29 as well as a hERG inhibitory activity of 78% at 5 µM (Table 1). Such profiles resulted in a lack of significant A $\beta$  reduction in mouse at 10 mg/kg and could raise

cardiovascular concerns if used clinically. Therefore, we initiated our efforts to improve the P-gp and hERG values in order to obtain centrally active BACE1 inhibitors with acceptable cardiovascular safety.

For central nervous system (CNS) drugs, optimal  $pK_a$  ranges of basic amines (the conjugate acids of amines), such as  $7.5 < pK_a < 10.5$  or  $pK_a < 8$ , were proposed.<sup>23</sup> According to these analyses, lowering  $pK_a$  achieved a decreased likelihood of P-gp recognition and hERG inhibition, while the lower limits were estimated based on data sets including compounds targeting monoamine receptors or transporters.<sup>23</sup> Considering this, we decided it would be important to determine the lower limit of  $pK_a$  utilizing BACE1 inhibitors alone, due to the difference in optimal  $pK_a$  ranges for retaining potency among these targets. Indeed, amine protonation is critical for inhibiting BACE1 enzyme at a given pH in the assay. Our BACE1 biochemical assay was conducted at pH 5.0, while our cellular assay was at the physiological pH of 7.4. Because the cellular potency reflects A $\beta$  reduction *in vivo*, understanding the lower limit of amidine pK<sub>a</sub> in our cellular assay was important for designing BACE1 inhibitors. For the analysis, we utilized our proprietary BACE1 inhibitors at that time, most of which included thiazine derivatives reported previously.<sup>25</sup> A good correlation between biochemical and cellular IC<sub>50</sub> values was observed except for compounds with a p $K_a < 6.5$  and a biochemical IC<sub>50</sub> <100 nM. The discrepancy came from the difference in pH used in our biochemical and cellular assays of 5.0 and 7.4, respectively. A box plot analysis using compounds with a biochemical IC<sub>50</sub> <100 nM clearly indicated that compounds with a p $K_a < 6.5$  reduced the cellular activities regardless of having good biochemical potency data (Supporting Information, Figure S1). Given that P-gp and the hERG channel recognize the ionization state at the physiological pH of 7.4, the optimal upper limit was

considered to be 7.4. The analysis and hypothesis led to a proposed guideline of  $6.5 < pK_a < 7.4$  for our research.

To develop BACE1 inhibitors meeting our  $pK_a$  guideline, we designed the oxazine head group **B** inserting a double bond into the dihydro-oxazine head group **A**. Because good alignment between the measured and ACD predicted  $pK_a$  values in **A** was observed, we utilized ACD software to predict the  $pK_a$  of **B**.<sup>26</sup> The result was an acceptable value of 7.0, most likely due to delocalization of the lone pairs on the nitrogen atoms, which initiated our efforts to synthesize the oxazine analogs.



**Figure 1.** Design of novel oxazine **B** from dihydro-oxazine **A**. <sup>*a*</sup>The p $K_a$  values were calculated with ACD/Percepta as the imine tautomer.<sup>26</sup>

**Optimization of the 1,3-Oxazine Head Group.** The designed compound **11** showed a reduced  $pK_a$  of 7.7 ( $\Delta pK_a$  of 2.1 over **6**) and an attenuated P-gp efflux ratio of 7.5, while exhibiting a slightly increased hERG inhibitory activity, 2-fold decrease in potency in the biochemical and cellular assays, and a significantly increased LogD when compared with **6** (Table 1). Additionally, we observed a discrepancy between the measured and predicted  $pK_a$ s (7.7 vs 7.0) lying outside the range of the guideline for  $pK_a$ . To mitigate hERG inhibitory activity as well as

improve potency, we investigated the incorporation of substituents on the double bond in 11. Compound 13, possessing a methyl group at the 6-position, was 3 times more potent than 11 in the biochemical assay, while moving the methyl group to the 5-position led to 12 with reduced potency, indicating that the 6-methyl oxazine 13 could serve as a key scaffold for fine-tuning the  $pK_a$  to within the optimal range. To further reduce the  $pK_a$ , we incorporated a fluorine atom at the 4-methyl group, leading to compound 14 with improved hERG liability. However, despite biochemical activity comparable to 13, decreased cellular potency was observed. This result can be explained by the  $pK_a$  of 6.3, which violates the lower limit of our analysis. Alternatively, substitution of the 6-methyl group in 13 with a fluorine led to compound 15 with an optimal  $pK_a$ of 6.9, which retained the potency associated with slightly reduced hERG inhibition relative to the 6-methyl 13. Replacement of the 4-methyl group with a cycloplopyl group (16) was not tolerated. Incorporation of methoxy as an alternative electron withdrawing group (17) led to a  $pK_a$  of 7.4. Although 17 had a  $pK_a$  value in the acceptable range, it showed reduced cellular potency and was found to be a strong P-gp substrate. On the other hand, its hERG potency was better than the fluorine analog 15 despite its higher  $pK_a$ . Although both the fluorine in 15 and the methoxy in 17 function as electron-withdrawing groups, the difference in hydrogen bonding capacity and lipophilicity between 15 and 17 can explain these discrepancies. The methoxy in 17 may have a favorable interaction with P-gp relative to the fluorine in 15, most likely because of its hydrogen bond accepting feature; in contrast, the more lipophilic fluorine in 15 may have better affinity for the hERG channel than the methoxy in **17**. Introduction of an additional fluorine in 15 led to 6-diffuromethyl 18 with a p $K_a$  of 6.4. Like compound 14, its reduced p $K_a$ was associated with impaired cellular potency. Consistent with the above analysis, such a low  $pK_a$  value was translated into improvements in P-gp and hERG levels. Except for 18 with a lower

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 $pK_a$  of 6.5, the compounds in Table 1 were more potent in the cellular assay than in the biochemical assay. This could be explained by accumulation of basic inhibitors in the acidic endosomes, where BACE1 resides and co-localizes with APP,<sup>4a</sup> and the pH is maintained in a range of 4.5 to 6.5.<sup>27</sup>

The rat pharmacokinetic (PK) profiles of selected analogs **6**, **14**, **15**, and **17** using our preliminary screening model (cassette dosing, n = 2) were assessed (Table 2). The initial lead **8** was stable in rat liver microsomes (RLM, 100% remaining after 30 min incubation), while a high fraction unbound in rat serum ( $f_{u,s}$  in rat) of 0.41 resulted in a moderate to high clearance of 37 mL/min/kg. Although **6** had a high Vd<sub>ss</sub> of 6.1 L/kg, a low total brain-to-plasma (B/P) ratio of 0.29, and an unbound brain-to-plasma ratio ( $K_{p,uu}$ ) of 0.057 were observed. Compounds **14** and **15** with reduced p $K_a$  values exhibited improved B/P ratios of 1.30 and 0.90 ( $K_{p,uu}$ s of 0.52 and 0.37), respectively, which can be explained by their reduced P-gp efflux ratios. Likewise, the reduced brain penetration of the methoxy analog **17** (B/P = 0.19) can be attributed to its high P-gp efflux. Despite their varied metabolic stability in RLM, all the compounds in Table 2 had favorable oral exposures. Taken together, 6-fluoromethyl **15** was notable in that it displayed a pronounced improvement in P-gp efflux resulting in a good B/P ratio as well as retaining cellular potency, although the hERG potency still required further mitigation.

# Table 1. Exploration of the Oxazine Head Group



<sup>*a*</sup>Values represent the mean values of at least two determinations. <sup>*b*</sup>Biochemical homogeneous time-resolved fluorescence (HTRF)-based assay. <sup>*c*</sup>IC<sub>50</sub> determined by measuring the levels of secreted Aβ40 in human APP-transfected human neuroblastoma (SH-SY5Y) cells via an HTRF-based assay. <sup>*d*</sup>Efflux ratio measured in MDCK cells transfected with human MDR1 at Absorption Systems. <sup>*e*</sup>% Inhibition at 5  $\mu$ M measured in HEK293 or CHO cells transfected with hERG channels using an automated patch clamp system. <sup>*f*</sup>LogD determined in 1-octanol/phosphate buffer at pH 7.4. <sup>*g*</sup>pK<sub>a</sub> determined by capillary electrophoresis (CE).

# Table 2. Pharmacokinetic Properties of Compounds 6, 14, 15 and 17 in Sprague-Dawley

|       | RLM              | fus/fubd     | rat, iv,                    | at, iv, 0.5 mg/kg, <i>n</i> = 2 <sup>a</sup> |                  |                                | rat, po                    | , 1 mg/kg, <i>n</i> = 2 <sup>l</sup>  | )         |
|-------|------------------|--------------|-----------------------------|--|------------------|--------------------------------|----------------------------|---------------------------------------|-----------|
| compd | (%) <sup>c</sup> | u,s · u,b    | CL (ml/min/kg) <sup>e</sup> | Vd <sub>ss</sub> (L/kg) <sup>f</sup>         | B/P <sup>g</sup> | K <sub>p,uu</sub> <sup>h</sup> | AUC (ng h/ml) <sup>i</sup> | C <sub>max</sub> (ng/ml) <sup>j</sup> | $F(\%)^k$ |
| 6     | 104              | 0.41 / 0.080 | 37.2                        | 6.1  | 0.29             | 0.057                          | 328                        | 32                                    | 73        |
| 14    | 38               | 0.14 / 0.056 | 20.6                        | 2.2  | 1.30             | 0.52                           | 395                        | 56                                    | 49        |
| 15    | 55               | 0.16 / 0.066 | 16.8                        | 3.4  | 0.90             | 0.37                           | 617                        | 77                                    | 52        |
| 17    | 61               | 0.13 / NT    | 12.9                        | 1.5  | 0.19             | ND                             | 892                        | 71                                    | 68        |

# **Rat Using a Cassette Method**

<sup>*a*</sup>Dosed as a suspension of test compounds in 0.5% methylcellulose (MC, 400 cP). <sup>*b*</sup>Dosed as a solution of test compounds in *N*,*N*-dimethylacetamide (DMA)/propylene glycol (PG) = 1/1. <sup>*c*</sup>% remaining in rat liver microsomes after 30 min incubation. <sup>*d*</sup> $f_{u,s}$  = Fraction unbound in rat serum.  $f_{u,b}$  = Fraction unbound in rat brain. NT = not tested. <sup>*e*</sup>Total clearance. <sup>*f*</sup>Volume of distribution at steady state. <sup>*g*</sup>Total brain-to-plasma ratio measured at 0.5 h. <sup>*h*</sup>Unbound brain-to-plasma ratio measured at 0.5 h. ND = not determined. <sup>*i*</sup>Plasma area under the concentration-time curve. <sup>*j*</sup>Maximal plasma concentration. <sup>*k*</sup>Oral bioavailability.

**Optimization of the Tail Group in 15.** In an effort to improve the hERG inhibitory activity of **15**, we focused on optimizing the tail group of R (Table 3). In addition to its suboptimal hERG profile, **15** was found to be somewhat unstable in rat plasma, relative to other compounds synthesized at our laboratory. Although this instability may be limited to the rat due to the highly expressed profile of carboxylesterases (CES) in the plasma compared to non-rodents,<sup>28</sup> we first investigated compounds with substituents at the *ortho*-position of the amide group such as **20**, **21**,

and **22**, in order to prevent hydrolysis by CES. As expected, these compounds were found to improve rat plasma  $t_{1/2}$  to more than 50 h. Interestingly, incorporating the *ortho*-substituents also reduced the hERG inhibitory activity relative to **15**, particularly in *ortho*-chloro **21** and *ortho*methoxy **22** (30% and 22% inhibition, respectively), while exhibiting an increased P-gp efflux which would lead to reduced brain penetration. Although the methyl **20** provided reduced hERG inhibition (50%) and a reasonable *B/P* ratio (0.62), decreased human liver microsomal (HLM) stability was observed compared to **15** (63% vs 88% remaining).

For further improvements in hERG as well as retaining acceptable ADMET profiles, a variety of 6-membered heterocycles was explored. Among them, pyrazine derivatives such as 23-26 were found to reduce hERG inhibitory activity. Although the methoxy pyrazine 23 had a reduced hERG inhibition of 30% at 5  $\mu$ M, it was associated with decreased plasma stability ( $t_{1/2}$  of 14.3 h) and poor RLM stability (13% remaining after 30 min) resulting in a high CL value of 64.9 ml/min/kg. Replacement of the methoxy group in 23 with difluoromethyl (24) or fluoromethoxy (25) provided molecules with improved RLM stability (44% and 52% remaining after 30 min, respectively), while these were still unstable in rat plasma (plasma  $t_{1/2} = 9.2$  h and 10.5 h, respectively). Furthermore, such analogs (23-25) displayed only modest potency in biochemical and cellular assays (cellular IC<sub>50</sub> = 22 nM, 72 nM, and 31 nM, respectively). To mitigate these problems observed with the pyrazine tails, a small set of substituents, such as methyl (data not shown), methoxy (data not shown), and amino, was incorporated on the pyrazine in 25 at the ortho-position of the amide group leading to aminopyrazine 26. Indeed, 26 was 5-fold more potent than the corresponding non-amino 25 (cellular IC<sub>50</sub> of 6.6 nM in 26), and plasma stability was also improved relative to the non-substituted 23, 24, and 25, but was comparable to the cyanopyridine 15 (plasma  $t_{1/2}$  of 22.3 h in 26). Despite adding the polar amino group, an

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increased LogD was unexpectedly observed for 26 in comparison to the non-amino 25, resulting in reduced metabolic stability in RLM and HLM (35% and 67% remaining after 30 min, respectively). However, although introduction of  $NH_2$  increased the hydrogen bond count, 26 provided a comparable P-gp efflux to the non-amino 25, very likely due to the intramolecular hydrogen bond of the amide carbonyl with the NH<sub>2</sub>. We postulated that the improved potency observed in 26 might come from the increased planarity between the amide and the pyrazine ring derived from the intramolecular hydrogen bond of the carbonyl with the amino group. Therefore, we hypothesized that reducing the ring size, which can maintain planarity and decrease lipophilicity, could lead to retaining potency as well as improving metabolic stability. Indeed, compounds with an oxazole ring such as 27 and 28 had a comparable cellular activity to the aminopyriazine 26. Like the pyrazines, changes to the isoxazoles retained the low hERG inhibitory potential. Unfortunately, despite their reduced LogD values relative to 26, the isoxazoles 27 and 28 still displayed suboptimal metabolic stability in RLM (57% and 39% remaining after 30 min, respectively). Furthermore, this modification was associated with increased P-gp recognition resulting in poor brain penetration. In the end, although the compounds in Table 2 did lower hERG inhibition compared to cyanopyridine 15, the improvement came at the cost of reduced potency, low metabolic stability, or high P-gp recognition. The balanced profile of **15** led to its being subjected to further study.

# Table 3. Exploration of the Oxazine Head Group



|                 |            | IC <sub>50</sub>   | (nM) <sup>a</sup>           |                         |                               |                             | rat F                          | PK <sup>g</sup>                             |                  |                             |
|-----------------|------------|--------------------|-----------------------------|-------------------------|-------------------------------|-----------------------------|--------------------------------|---|------------------|-----------------------------|
| compd           | R          | BACE1 <sup>b</sup> | Cellular<br>Aβ <sup>c</sup> | P-gp<br>ER <sup>d</sup> | hERG<br>5 µM (%) <sup>e</sup> | RLM<br>HLM (%) <sup>f</sup> | CL<br>(ml/min/kg) <sup>h</sup> | Plasma<br>t <sub>1/2</sub> (h) <sup>i</sup> | B/P <sup>i</sup> | Log D<br>(7.4) <sup>k</sup> |
| 15              | NC         | 59.0               | 3.6                         | 12                      | 69                            | 55<br>88                    | 16.8                           | 20.4  | 0.90             | 2.3                         |
| 20              | NC         | 66.6               | 8.4                         | 18                      | 50                            | 34<br>63                    | 21.8                           | 55.3  | 0.62             | 2.5                         |
| 21              | NC N<br>CI | 94.9               | 12                          | 35                      | 30                            | 49<br>57                    | 30.6                           | 56.7  | 0.41             | 2.5                         |
| 22              | NC N       | 238                | 56                          | 44                      | 22                            | 60<br>85                    | 36.6                           | 69.4  | 0.089            | 1.9                         |
| 23              |            | 241                | 22                          | 2.7                     | 30                            | 13<br>80                    | 64.9                           | 14.3  | 2.9              | 2.7                         |
| 24              |            | 313                | 72                          | 8.5                     | 33                            | 44<br>84                    | 48.1                           | 9.20  | 2.7              | 2.5                         |
| F∖<br><b>25</b> |            | 139                | 31                          | 5.7                     | 40                            | 52<br>86                    | 34.4                           | 10.5  | 2.4              | 2.6                         |
| F⊾<br>26        |            | 34.8               | 6.6                         | 4.9                     | 20                            | 35<br>67                    | 45.3                           | 22.3  | 2.6              | 3.1                         |
| 27              | F          | 94.1               | 6.5                         | 25                      | 35                            | 57<br>84                    | 28.4                           | 9.30  | 0.30             | 2.6                         |
| 28              | F          | 48.4               | 5.8                         | 16                      | 18                            | 39<br>76                    | 55.0                           | 26.7  | 0.38             | 2.2                         |



<sup>*a*</sup>Values represent the mean values of at least two determinations. <sup>*b*</sup>Biochemical homogeneous time-resolved fluorescence (HTRF)-based assay. <sup>*c*</sup>IC<sub>50</sub> determined by measuring the levels of secreted Aβ40 in human APP-transfected human neuroblastoma (SH-SY5Y) cells via an HTRF-based assay. <sup>*d*</sup>Efflux ratio measured in MDCK cells transfected with human MDR1 at Absorption Systems. <sup>*e*</sup>% inhibition at 5 µM measured in CHO cells transfected with hERG channels using an automated patch clamp system. <sup>*f*</sup>% Remaining after 30 min incubation with rat liver (RLM) and human liver microsomes (HLM). <sup>*g*</sup>Sprague-Dawley rats. <sup>*h*</sup>Total clearance dosed *iv* at 0.5 mg/kg as a solution in DMA/PG = 1/1 (*n* = 2). <sup>*i*</sup>Plasma half-life *in vitro*. <sup>*j*</sup>Total brain-to-plasma ratio determined by *iv* dose at 0.5 h. <sup>*k*</sup>LogD determined in 1-octanol/phosphate buffer at pH 7.4.

**Cocrystal Structure of 15 Bound to BACE1.** The X-ray structure of **15** complexed with human BACE1 was determined at a resolution of 2.30 Å (Figure 2). Compound **15** bounds to a flapclosed BACE1 structure, in which the Tyr71 side chain hydroxyl on the flap forms a hydrogenbond interaction with the NH of the Trp76 side chain. Like other amidine-based inhibitors,<sup>8,9</sup> the amidine moiety of **15** interacts with the two catalytic aspartate residues 32 and 228. The two aromatic rings of the fluorophenyl and the cyanopyridine occupy the S1 and S3 pockets of the enzyme, respectively. In addition, the fluorine on the phenyl forms a van der Waals contact with the side chain of Tyr71. Indeed, the corresponding non-fluorine analog was 2–3 times less potent than **15** in the biochemical assay (data not shown) indicating the importance of the fluorine. Finally, the amide NH hydrogen forms a hydrogen bond with the backbone carbonyl oxygen of Gly230 as well as with the pyridine nitrogen in **15**.



**Figure 2.** Cocrystal structure of the oxazine **15** bound to the active site of human BACE1 determined at 2.30 Å resolution (PDB: 5YGY). Purple mesh shows |Fo| - |Fc| map contoured at 2.0  $\sigma$  in which ligand coordinates are omitted from the calculation. Key residues of the catalytic residues Asp228 and Asp32 (green), Tyr71 (purple) on the Flap region, and Gly230 (gray) are shown in stick style. Compound **15** is shown in ball-and-stick style with carbon atoms colored yellow. Nitrogen, oxygen, and fluorine atoms are colored blue, red, and sky blue, respectively. Dashed lines represent hydrogen bond interactions between **15** and BACE1.

In vitro ADMET Profiles of 15. The free base of compound 15 formed a crystalline powder that had good aqueous thermodynamic solubility of 3220 µg/mL and 28.7 µg/mL at pH 1.2 and 6.8, respectively. Consistent with the favorable LogD at pH 7.4 of 2.3, the permeability in wildtype MDCK cells was in an acceptable range for CNS drugs ( $P_{app} = 20.4 \times 10^{-6}$  cm/s). Prior to PK studies in multiple species, liver microsomal stability and the fraction unbound in serum ( $f_{u,s}$ ) were determined. Like the stability in HLM, compound 15 was found to be stable in mouse, dog, and monkey microsomes (69%, 87%, and 79% remaining after 30 min, respectively), and its  $f_{u,s}$ 

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was moderate to high in rat, mouse, dog, monkey, and guinea-pig (0.16, 0.30, 0.11, 0.22, and 0.42, respectively). Brain tissue binding in rat was found to be moderate ( $f_{ub} = 0.066$ ) as shown in Table 2. Compound 15 was also evaluated for its ability to inhibit a panel of cytochrome P450s and exhibited IC<sub>50</sub>s of more than 20 µM over 1A2, 2C9, 2C19, 2D6 and 3A4. Additionally, 15 did not show time-dependent inhibition of CYP3A4 up to 20  $\mu$ M. The hERG IC<sub>50</sub> value using a manual patch clamp method was determined to be 2.0  $\mu$ M. The close analog 4-fluoromethyl 14 exhibited hERG IC<sub>50</sub> of 3.9  $\mu$ M reflecting the difference in % inhibition at 5  $\mu$ M. Compound 15 was tested against other aspartyl proteases, cathepsin D (CatD) and BACE2. Although 15 showed limited selectivity over BACE2 ( $IC_{50} = 86.3 \text{ nM}$ ), no significant inhibition was observed in CatD (IC<sub>50</sub> >100  $\mu$ M). Solid state stability testing for 15 was conducted at 40 °C/89% relative humidity (RH), where a degradation product increased by 0.06%, indicating that 15 was almost stable. Because 15 has a potential to generate anilines as metabolites (a structural alert), the Ames test was conducted to estimate the genetic toxicity of this series. Five mutant strains of TA100, TA1535, TA1537, TA98, and WP2uvrA were used in the presence and absence of the rat S9 fraction. Negative results were observed for 15 at doses up to 5 mg/plate. Also, the corresponding aniline dihydrochloride was found to be negative in the Ames test.<sup>29</sup>

**Brain Penetration in Wild-Type and Mdr1a Knockout Mouse.** From exploration of the head group, compound **15** provided lower efflux ratios in MDCK cells transfected with human MDR1 relative to the initial lead **6**. To investigate the potential impact of P-gp *in vivo*, we utilized *mdr1a* knockout (KO) mouse to determine *B/P* ratios in knockout versus wild-type (WT). For comparison, oxazine **14** with a lower P-gp efflux of 9.0 and dihydro-oxazine **6** with a higher P-gp efflux of 36 were included in the tests. The compounds were administered orally at doses of 2 and 10 mg/kg (2 mg/kg for **6** and **15**, 10 mg/kg for **14**). Clearly, the P-gp efflux ratios were

translated into the KO/WT ratios of the *B/P* values. As shown in Table 4, the *B/P* ratios in WT mouse were comparable to those in rat in Table 2, while increased *B/P* ratios were observed in P-gp KO mouse, indicating that these compounds were P-gp substrates in mouse. Despite the low lipophilicity of the initial lead 6 (LogD = 0.024) and the low permeability ( $P_{app} = 0.32 \times 10^{-6}$  cm/s), the *B/P* ratio in the KO mouse significantly increased to 4.7 (from 0.15 in the WT counterpart), which highlighted the important role of P-gp in brain penetration. The *B/P* ratios in the KO mouse for 14 and 15 were 2.5 and 4.4 resulting in KO/WT ratios of 2.5 and 3.6, respectively, demonstrating the improvement in P-gp efflux for 14 and 15 *in vivo* relative to 6 (KO/WT = 32). According to the analysis at Pfizer for successful CNS drugs using P-gp KO mouse,<sup>30</sup> KO/WT ratios of 2 to 3 appeared to be in an acceptable range. Thus, although both 14 and 15 were still found to be P-gp substrates in mouse, the magnitude of the potential clinical effects for 14 and 15 may be low.

 Table 4. Brain Penetrations of 6, 14, and 15 in Wild-type (C57BL/6J) and mdr1a (-/-)

 Mouse.

|       |                         | po, 2 or 10 mg/kg, <i>n</i> = 3 <sup>a</sup> |                    |                                       |  |                  |                    |
|-------|-------------------------|--|--------------------|---------------------------------------|--|------------------|--------------------|
| compd | P-gp<br>ER <sup>b</sup> | dose<br>(mg)                                 | mouse <sup>c</sup> | C <sub>b</sub><br>(ng/g) <sup>d</sup> | C <sub>p</sub><br>(ng/ml) <sup>e</sup> | B/P <sup>f</sup> | KO/WT <sup>g</sup> |
| 6     | 36                      | 2  | WT                 | 14.5                                  | 102                                    | 0.15             | 32                 |
|       |                         |  | KO                 | 546                                   | 118                                    | 4.7              |                    |
| 14    | 9.0                     | 10   | WT                 | 341                                   | 336                                    | 1.0              | 2.5                |
|       |                         |  | KO                 | 903                                   | 363                                    | 2.5              |                    |
| 15    | 12                      | 2  | WT                 | 92.6                                  | 76.1                                   | 1.2              | 3.6                |
|       |                         |  | KO                 | 318                                   | 71.5                                   | 4.4              |                    |

<sup>*a*</sup>Dosed as a suspension of test compounds in 0.5% methylcellulose (MC). <sup>*b*</sup>Efflux ratio measured in MDCK cells transfected with human MDR1 at Absorption Systems. <sup>*c*</sup>KO = mdr1a (-/-) mouse; WT = wild-type (C57BL/6J) mouse (vehicle = 0.5% MC). <sup>*d*</sup>Brain concentration at 2 h

time point. <sup>*e*</sup>Plasma concentration at 2 h time point. <sup>*f*</sup>Brain-to-plasma total concentration ratio  $(C_b/C_p)$ . <sup>*g*</sup>KO/WT = (B/P in KO)/(B/P in WT).

Neuro-Pharmacokinetic Studies of 15 in Multiple Species. Investigations of pharmacokinetic properties of 15 were conducted using Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. As shown in Table 5, exposure and plasma levels were high in rats after oral administration of 15 at doses of 1, 3, and 10 mg/kg. At 1 mg/kg, the maximum concentration  $(C_{\text{max}})$  and the area under the curve (AUC) were at similar levels to those in the cassette method (Table 5 vs Table 2). Also, the intravenous administration at 2 mg/kg provided comparable results to those from the cassette method of 0.5 mg/kg. The dose-proportionality of 15 was maintained between the given doses. Compound 15 showed high exposure, moderate clearance, and moderate to high volume of distribution. To assess neuro-pharmacokinetic profiles of 15, compound levels in the brain and CSF were also measured at the po dose of 3 mg/kg (Table 6). Total *B/P* ratios ( $C_b/C_p$ , 0.70 to 0.83) were similar to unbound brain-to-plasma ratios based on CSF levels ( $C_{\text{CSF}}/C_{\text{p,u}}$ , 0.51 to 0.78), while those based on unbound brain levels ( $C_{\text{b,u}}/C_{\text{p,u}}$ ;  $C_{\text{b,u}}$  =  $C_{\rm b} \times f_{\rm u,b}$ ; 0.29 to 0.34) decreased 2-fold relative to the total B/P and  $C_{\rm CSF}/C_{\rm p,u}$  ratios. The higher  $C_{\rm CSF}/C_{\rm p,u}$  ratio could be rationalized by the observed P-gp effect in mouse, because P-gp works as an active transporter at the blood-CSF barrier.<sup>23</sup>

Table 7 summarizes the pharmacokinetic data for beagle dog and cynomolgus monkey. In dog, **15** displayed low clearance, moderate to high  $Vd_{ss}$ , a decent exposure level (AUC), and high bioavailability following an *iv* dose of 1 mg/kg and a *po* dose of 0.75 mg/kg (day 1). After repeated daily *po* doses, slight increases in  $C_{max}$  and AUC were seen on day 7. In contrast to dog, a higher clearance and decreased bioavailability were observed in monkey, most likely due to

decreased microsomal stability and higher  $f_{u,s}$  as shown in Table 7. To compare rat and dog CNS availability, compound levels in CSF were measured at 10 and 24 h after dosing on day 7, where comparable  $C_{CSF}/C_{p,u}$  ratios were obtained between rat and dog. On the basis of the good brain penetration observed, **15** was subjected to pharmacokinetic/pharmacodynamics (PK/PD) study.

|      |                                       | rat, po, <i>n</i> = 3 <sup>a</sup> |                 |                            |
|------|---------------------------------------|------------------------------------|-----------------|----------------------------|
| dose | C <sub>max</sub> (ng/ml) <sup>b</sup> | AUC (ng∙h/ml) <sup>c</sup>         | F(%             | ) <sup>d</sup>             |
| 1    | 70.0 ± 9.0                            | 358 ± 20                           | 33              |                            |
| 3    | 274 ± 22                              | 2040 ± 570                         | 62              |                            |
| 10   | 965 ± 233                             | 4990 ± 1000                        | 46              |                            |
|      |                                       | rat, iv, <i>n</i> = 3 <sup>e</sup> |                 |                            |
| dose | CL (ml/min/kg) <sup>f</sup>           | $Vd_{ss} (L/kg)^g t_{1,s}$         | /2 <sup>h</sup> | AUC (ng·h/ml) <sup>i</sup> |
| 2    | 15.4 ± 2.1                            | 2.93 ± 0.33 2.45 :                 | ± 0.53          | 2190 ± 300                 |

Table 5. Pharmacokinetic Properties of 15 at Multiple Doses in Rat

<sup>*a*</sup>Dosed as a suspension of **15** in 0.5% MC. <sup>*b*</sup>Maximal plasma concentration. <sup>*c*</sup>Plasma area under the curve (*po*). <sup>*d*</sup>Oral bioavailability. <sup>*e*</sup>Dosed as a solution of **15** in 0.5% MC. <sup>*f*</sup>Total clearance. <sup>*g*</sup>Volume of distribution at steady state. <sup>*h*</sup>Half-life (*iv*). <sup>*i*</sup>Plasma area under the curve (*iv*).

Table 6. CNS Availability Data for 15 in Rat

|                                  | _                         | time after administration / po, 3 mg/kg, $n = 3^a$ |                      |                       |                       |  |  |
|----------------------------------|---------------------------|--|----------------------|-----------------------|-----------------------|--|--|
|                                  |                           | 1 h  | 3 h                  | 5 h                   | 7 h                   |  |  |
| C <sub>p</sub> (ng/r             | nl) <sup>b</sup> 7        | 70.0 ± 9.0   | 51.0 ± 5.8           | 19.5 ± 4.7            | 19.6 ± 4.0            |  |  |
| C <sub>b</sub> (ng/g             | ) <sup>c</sup> 4          | 18.9 ± 1.6   | 42.2 ± 8.4           | 16.1 ± 5.8            | 15.5 ± 4.0            |  |  |
| C <sub>b,u</sub> (ng             | /g) <sup>d</sup> 3        | 3.2 (8.4 nM)                                       | 2.8 (7.2 nM)         | 1.1 (2.8 nM)          | 1.0 (2.7 nM)          |  |  |
| C <sub>CSF</sub> (ng             | g/ml) <sup>e</sup> 7<br>( | 7.5 ± 1.2<br>20 nM)                                | 4.9 ± 0.4<br>(13 nM) | 2.4 ± 1.4<br>(6.3 nM) | 1.6 ± 0.4<br>(4.2 nM) |  |  |
| $C_{\rm b}/C_{\rm p}^{f}$        |                           | 0.70   | 0.83                 | 0.83                  | 0.79                  |  |  |
| $C_{\rm b,u}/C_{\rm p,u}$        | g<br>u                    | 0.29   | 0.34                 | 0.34                  | 0.32                  |  |  |
| C <sub>CSF</sub> /C <sub>p</sub> | h<br>o,u                  | 0.67   | 0.61                 | 0.78                  | 0.51                  |  |  |

<sup>*a*</sup>Dosed as a suspension of **15** in 0.5% MC. <sup>*b*</sup>Plasma concentration. <sup>*c*</sup>Brain concentration. <sup>*d*</sup>Unbound brain concentration ( $C_{b,u} = C_b \times f_{u,b}$ ). <sup>*d*</sup>Cerebrospinal fluid (CSF) concentration. <sup>*e*</sup>Total brain-to-plasma ratio (B/P or  $K_p$ ). <sup>*f*</sup>Unbound brain-to-plasma ratio ( $K_{p,uu}$ ).  $C_{b,u} = C_b \times f_{u,b}$ (unbound brain fraction in rat).  $C_{p,u} = C_p \times f_{u,s}$  (unbound plasma fraction in rat). <sup>*g*</sup>CSF-tounbound plasma ratio ( $K_{p,uu}$  (CSF)).

| Table 7. | Pharmacol   | kinetic Pro | perties of 15 | in Beagle I  | Dog and C | vnomolgus   | Monkev |
|----------|-------------|-------------|---------------|--------------|-----------|-------------|--------|
| I upic / | I mai macoi | mette i i o |               | III Dougio I |           | y nomongus. | Juney  |

|                            | LM               | fus              | iv, 1 r                     | ng/kg, <i>n</i> = 3 <sup>c</sup>     |               | po, 0.75 mg/kg (dog), 1 mg/kg (monkey), $n = 3^{g}$ |
|----------------------------|------------------|------------------|-----------------------------|--------------------------------------|---------------|---|
| species                    | (%) <sup>a</sup> | (%) <sup>b</sup> | CL (ml/min/kg) <sup>d</sup> | Vd <sub>ss</sub> (L/kg) <sup>e</sup> | $t_{1/2}^{f}$ | AUC $(ng \cdot h/ml)^h C_{max} (ng/ml)^i F (\%)^j$  |
| dog<br>(dav1)              | 87               | 0.11             | 5.95 ± 1.4                  | 2.66 ± 0.69                          | 5.75 ± 0.2    | 28 2403 ± 644 173 ± 24.9 91                         |
| dog<br>(day7) <sup>k</sup> |                  |                  | NT                          | NT                                   | NT            | 2722 ± 743 198 ± 31.8 ND                            |
| monkey                     | 79               | 0.22             | 20.8 ± 5.3                  | 5.01 ± 1.09                          | 5.48 ± 0.3    | 31 924 ± 173 92.9 ± 30.2 37                         |

<sup>*a*%</sup> remaining in dog or monkey liver microsomes after 30 min incubation. <sup>*b*</sup>Fraction unbound in dog or monkey serum. <sup>*c*</sup>Dosed as a solution of **15** in DMA/PG/ hydroxypropyl- $\beta$ -cyclodextrin (HPBCD) = 1/3/1 for dog and in HPBCD for monkey. NT = not tested. <sup>*d*</sup>Total clearance. <sup>*e*</sup>Volume of distribution at steady state. <sup>*f*</sup> Half-life (*iv*). <sup>*g*</sup>Dosed as a suspension of **15** in 0.5% MC. <sup>*h*</sup>Plasma area under the curve (*po*). <sup>*i*</sup>Maximal plasma concentration. <sup>*j*</sup>Oral bioavailability. ND = not determined. <sup>*k*</sup>After 7 days of once daily dosing, plasma samples were collected for up to 24 h.

# Table 8. CNS Availability Data For 15 in Dog

| po (day7), 0.75 mg/kg, <i>n</i> = 3 <sup>a</sup> |                    |               |   |         |               |               |                           |  |
|--|--------------------|---------------|---|---------|---------------|---------------|---------------------------|--|
| 10 h, ng/ml                                      |                    |               |   |         | 24            | h, ng/r       | nl                        |  |
| Cp <sup>b</sup>                                  | C <sub>p,u</sub> c | $C_{CSF}^{d}$ | C <sub>CSF</sub> /C <sub>p,u</sub> <sup>e</sup> | $C_{p}$ | $C_{\rm p,u}$ | $C_{CSF}$     | $C_{\rm CSF}/C_{\rm p,u}$ |  |
| 107  | 11.8               | 6.8<br>(18 nl | 0.58<br>VI)                                     | 27.7    | 3.05          | 1.7<br>(4.4 r | 0.56<br>nM)               |  |

<sup>*a*</sup>After 7 days of once daily dosing (po, 0.5% MC), cisternal CSF samples were collected from anesthetized dogs at 10 and 24 h time points. <sup>*b*</sup>Plasma concentration measured in the experiment in Table 7. <sup>*c*</sup>Unbound plasma concentration ( $C_{p,u} = C_p \times f_{u,s}$  (unbound plasma fraction in dog)). <sup>*d*</sup>Cerebrospinal fluid (CSF) concentration. <sup>*e*</sup>CSF-to-unbound plasma ratio ( $K_{p,uu}$  (CSF)).

**PK/PD Studies of 15 in Mouse and Dog.** To assess BACE1 inhibition *in vivo*, we used Crlj:CD (ICR) mouse<sup>31</sup> and beagle dog. As shown in Figure 3, **15** demonstrated robust and sustained reduction of total Aβ levels in the brain over 8 h following a *po* dose of 10 mg/kg. The maximum Aβ reduction of 69% was observed at 4 h, where the brain level reached 295 ng/mL ( $C_{b,u} = 51$  nM), which was 14-fold higher than a cellular IC<sub>50</sub> value of 3.6 nM. Even at 8 h, **15** achieved 1.9-fold higher unbound brain level than the IC<sub>50</sub>, explaining the sustained Aβ reduction in mouse.



**Figure 3.** Total A $\beta$  reduction in mouse after an oral dose of **15** at 10 mg/kg (n = 4). Compound **15** was dosed as a solution of 20% 2-hydroxypropyl- $\beta$ -cyclodextrin (HPBCD).

Dog models for assessing the potential of inhibiting A $\beta$  production are useful intermediates between models for mice and humans, because pathological changes of A $\beta$  production in dogs are similar to those in humans, and dogs also develop A $\beta$  plaques thereby being considered as a natural model of age-dependent cognitive dysfunction.<sup>32</sup> To determine the plasma level that can achieve A $\beta$  reduction by 50% (EC<sub>50</sub>), a PK/PD study in dog was conducted at multiple doses of

0.31, 1.25, and 5 mg/kg.<sup>32a</sup> The plasma samples were taken at 0.5, 1, 2, 4, 8, and 25 h after dosing, and the CSF was sampled in conscious dogs from the lateral ventricle before dosing and at 4, 8, and 25 h after dosing. As shown in Figure 4, **15** demonstrated a dose-dependent and sustained decrease in CSF A $\beta$  40 and 42. Importantly, even at 1.25 mg/kg, a robust CSF A $\beta$ reduction of more than 70% was achieved at 8 h. Finally, the EC<sub>50</sub> value in plasma was determined to be 62 ng/mL (EC<sub>50, unbound</sub> = 18 nM); the EC<sub>50</sub> value in CSF was estimated to be 10 nM using the *C*<sub>CSF</sub>/*C*<sub>p,u</sub> ratios of 0.56 in Table 8, which was 3-fold higher than the cellular IC<sub>50</sub> value of 3.6 nM. For comparison, a dog PK/PD study for **14** was also done, and the EC<sub>50</sub> value for A $\beta$ 42 was found to be 145 ng/mL (EC<sub>50, unbound</sub> = 45 nM, *f*<sub>u,s</sub> in dog = 0.12), reflecting the difference in the cellular IC<sub>50</sub> values between **14** and **15** of 17 nM and 3.6 nM, respectively.



**Figure 4.** CSF A $\beta$ 1–40, and A $\beta$ 1–42 reduction in beagle dog after oral doses of **15** at 0.31, 1.25, and 5 mg/kg (*n* = 4). Compound **15** was dosed as a solution of 20% HPBCD.

**Cardiovascular Safety of 15 in Anesthetized Guinea-Pig and Conscious Dog.** To investigate the cardiovascular profile of **15**, we first utilized an anesthetized guinea-pig model. Increasing doses of **15** (3, 10, and 30 mg/kg) were administered intravenously over a period of 10 min at 30 min intervals to urethane-anesthetized guinea-pigs. The average unbound plasma levels of **15**,

measured at the end of the intravenous infusions of 3, 10, and 30 mg/kg, were 0.907, 4.31, and 13.4  $\mu$ M, respectively. Compared to the vehicle, **15** significantly increased the duration of QTc interval (13.9%) and decreased the heart rate at 30 mg/kg. The unbound no-observed-adverse-effect level (NOAEL) for cardiovascular safety in guinea-pig is 4.31 $\mu$ M, and the safety margin over the dog EC<sub>50,unbound</sub> was calculated to be 239-fold—wide safety margin.

The cardiovascular effects of **15** in conscious dogs were then studied. On the basis of the dog PK study in Table 7 and the NOAEL in guinea-pigs, **15** was orally administered at doses of 30 and 100 mg/kg. No significant effects on arterial blood pressure, heart rate, PR interval, and QTc interval were observed at 30 mg/kg, while statistically significant increases in heart rate and QTc interval (16.1%) were observed at 100 mg/kg. The unbound maximum concentrations ( $C_{max,unbound}$ ) for 30 and 100 mg/kg were 1.40 and 3.21 µM, respectively; **15** had a NOAEL of 1.40 µM, which was close to the hERG IC<sub>50</sub> value of 2.0 µM, establishing the safety margin of 78-fold over dog EC<sub>50,unbound</sub>. Although the margin was lower than observed for guinea pigs, it was still in an acceptable range.

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The synthesis of oxazine **11** is outlined in Scheme 1. Condensation of acetophenone **30** with (*R*)*tert*-butysulfinamide<sup>33</sup> using Ti(OEt)<sub>4</sub> afforded ketimine **31**. This was followed by reduction of the nitro group and trifluoroacetylation with trifluoroacetic anhydride (TFAA) to provide **32**. Addition of allyl Grignard reagent to the ketimine **32** afforded compound **33** as a single diastereomer, which was followed by ozonolysis to give aldehyde **34**. The *tert*-butylsulfinyl group in **34** was removed with HCl in MeOH to give acetal **35**, which was then reacted with triphosgene followed by diallylamine, yielding urea **36**. The oxazine ring was formed using

Burgess reagent<sup>34</sup> in the presence of pyridinium *p*-toluenesulfonate (PPTS) to provide **37**. Deprotection of the trifluoroacetyl group in **37** and amide coupling with 5-cyanopicolinic acid followed by deprotection of the diallyl group in **39** in the presence of palladium catalyst afforded the oxazine **11**. The stereochemistry of **11** was confirmed by single crystal X-ray analysis of compound **18**, which was synthesized via the intermediate **33** (see Supporting Information).

Scheme 1. Synthesis of Oxazine 11<sup>a</sup>



<sup>*a*</sup>Reagents and conditions: (a) (*R*)-2-methylpropane-2-sulfinamide, Ti(OEt)<sub>4</sub>, THF, 65 °C, 65%; (b) (i) Fe, NH<sub>4</sub>Cl, toluene–H<sub>2</sub>O, 80 °C, (ii) TFAA, Et<sub>3</sub>N, THF, –25 °C, 78%; (c) allylmagnesium bromide, THF, –78 °C, 40%; (d) O<sub>3</sub>, DCM, –78 °C, 88%; (e) 2 N HCl in MeOH, rt, 56%; (f) triphosgene, Et<sub>3</sub>N, diallylamine, H<sub>2</sub>O–EtOAc, 0 °C, 82%; (g) (i) aq H<sub>2</sub>SO<sub>4</sub>, acetone, (ii) Burgess reagent, PPTS, THF, reflux, 24%; (h) K<sub>2</sub>CO<sub>3</sub>, THF–MeOH–H<sub>2</sub>O, 40 °C, 87%; (i) EDC, HOBt,

DMAP, 5-cyanopicolinic acid, DMF, rt, 92%; (j) 1,3-dimethylbarbituric acid, Pd(PPh<sub>3</sub>)<sub>4</sub>, DCM, reflux, 58%.

The 6-fluoromethyl substituted oxazines 15 and 20-28 were synthesized via the route outlined in Scheme 2. Sulfinyl ketimine 40, prepared from the commercially available 2-fluoroacetophenone, was reacted with allyl Grignard reagent to provide compound 41 as a single diastereomer, which was followed by cleavage of the sulfinyl group to afford allyl amine 42. Urea formation was done with 4-nitrophenyl chloroformate and bis(2,4-dimethoxybenzyl)amine to give urea 43, which was then oxidized to the hydroxy ketone 44. Use of 4-nitrophenyl chloroformate, instead of triphosgene, under the Schotten-Baumann reaction condition avoided formation of a side product affording 43 with improved yield. Unlike 36, cyclization of the ketone 43 was accomplished without PPTS using Burgess reagent, furnishing oxazine 45. Replacement of the mesylate group with NaI to 46 followed by fluorination using AgF led to 6-fluoromethyl oxazine 47. After cleavage of the bis-2.4-dimethoxybenzyl group using TFA, 48 was subjected to nitration reaction, which was followed by reduction of the nitro group and subsequent Boc protection resulting in aniline 51. Finally, amide formation followed by deprotection delivered the 6-fluoromethyl substituted oxazines 15 and 20–28. The absolute configuration was confirmed by the X-ray structure of 15 bound to BACE1 (Figure 2).

#### Scheme 2. Synthesis of Oxazines 15 and 20–28<sup>a</sup>



<sup>*a*</sup>Reagents and conditions: (a) allylmagnesium bromide, Et<sub>2</sub>O, -60 to -20 °C, 72%; (b) 4 N HCl in EtOAc, MeOH, 0 °C, quant; (c) 4-nitrophenyl chloroformate, K<sub>2</sub>CO<sub>3</sub>, bis(2,4-dimethoxybenzyl)amine, EtOAc-H<sub>2</sub>O, 0 °C to rt, 82%; (d) KMnO<sub>4</sub>, AcOH, acetone-H<sub>2</sub>O, rt, 68%; (e) (i) Ms<sub>2</sub>O, DCM, 0 °C, (ii) Burgess reagent, THF, rt, 40%; (f) NaI, acetone, rt, 95%; (g) AgF, MeCN, rt, 98%; (h) anisole, TFA, 80 °C, quant; (i) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>-TFA, -20 °C, 94%; (j) Boc<sub>2</sub>O, DMAP, THF, 0 °C, 90%; (k) Fe, NH<sub>4</sub>Cl, EtOH-THF-H<sub>2</sub>O, 60 °C, 85%; (l) (i) HATU, RCO<sub>2</sub>H, DIEA, DCM, rt, (ii) HCO<sub>2</sub>H, rt, 61–95%.

# CONCLUSION

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Lead optimization efforts on the dihydro-oxazine 6 led to the discovery of a series of oxazines exemplified by 15 with improved P-gp efflux resulting in robust A $\beta$  reduction in the brain. We employed a  $pK_a$  lowering approach on the amidine moiety by incorporating a double bond in an attempt to mitigate the high P-gp efflux observed in 6. This led to identification of oxazine 11 with improved P-gp efflux, which we believe is the first report of using a double bond to reduce the  $pK_a$  of basic amines for BACE1 inhibitors. Subsequent optimization of the head group followed by the tail culminated in 15 as the best in the series. The P-gp knockout mouse study suggested that the significant improvement in brain penetration observed in 15 relative to 6 resulted from the reduced P-gp efflux. The decent unbound B/P ratios as well as adequate target coverage were confirmed with the rat and dog neuroPK studies. Reflecting CNS availability, 15 displayed a significant A $\beta$  reduction in mouse at 10 mg/kg and a low A $\beta$ 42 EC<sub>50</sub> value of 62 ng/mL in dog. While 15 still inhibited the hERG channel at 5  $\mu$ M, wide safety margins over the dog EC<sub>50</sub> were demonstrated in the cardiovascular safety models. Finally, the well characterized 15 using neuro-pharmacokinetic models, a P-gp KO mouse model, and *in vivo* cardiovascular models should serve as a useful tool for finding BACE1 inhibitors.

#### **EXPERIMENTAL SECTION**

**General Chemistry.** All commercial reagents and solvents were used as received unless otherwise noted. Thin layer chromatography (TLC) analysis was performed using Merck silica gel 60  $F_{254}$  thin layer plates (250 µm in thickness). Flash column chromatography was carried out on an automated purification system using Yamazen or Fuji Silysia prepacked silica gel columns. <sup>1</sup>H NMR spectra were recorded on a Bruker Advance 400 MHz. Spectral data are reported as follows: chemical shift (as ppm referenced to tetramethylsilane), multiplicity (s = singlet, d = doublet, dd = double doublets, dt = double triplet, t = triplet, q = quartet, m =

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multiplet, br = broad peak), coupling constant, and integration value. Analytical LC/MS was performed on a Shimadzu Shim-pack XR-ODS ( $C_{18}$ , 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 = H<sub>2</sub>O + 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 on a Thermo Fisher Scientific LTQ Orbitrap using electrospray positive ionization.

#### (S)-N-(3-(2-Amino-4-methyl-4H-1,3-oxazin-4-yl)-4-fluorophenyl)-5-cyanopicolinamide (11).

To a solution of **39** (66.9 mg, 0.155 mmol) and 1,3-dimethylbarbituric acid (145 mg, 0.930 mmol) in DCM (1.0 mL) was added Pd(PPh<sub>3</sub>)<sub>4</sub> (17.9 mg, 0.016 mmol). The mixture was refluxed for 2 h. The mixture was diluted with EtOAc and saturated aqNaHCO<sub>3</sub> solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with H<sub>2</sub>O and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (amino silica gel; hexane/EtOAc, gradient: 20–50% EtOAc) to give **11** (32 mg, 58% yield) as a white solid. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.56 (s, 3H), 4.11 (br s, 2H), 5.56 (dd, *J* = 6.1, 2.5 Hz, 1H), 6.37 (d, *J* = 6.1 Hz, 1H), 7.06 (dd, *J* = 11.2, 8.6 Hz, 1H), 7.67 (dd, *J* = 4.1, 3.0 Hz, 1H), 7.91–7.95 (m, 1H), 8.20 (dd, *J* = 8.1, 2.0 Hz, 1H), 8.43 (d, *J* = 8.1 Hz, 1H), 8.89 (d, *J* = 6.1, 2.0 Hz, 1H), 9.85 (s, 1H). MS-ESI (*m/z*): 352 [M + H]<sup>+</sup>.

(*S*)-*N*-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl)-4-fluorophenyl)-5cyanopicolinamide (15). To a solution of 51 (10.3 g, 22.7 mmol), DIEA (7.92 mL, 45.3 mmol), and 5-cyanopicolinic acid monohydrate (4.52 g, 27.2 mmol) in DCM (100 mL) was added

HATU (10.3 g, 27.2 mmol) at room temperature, and the reaction mixture was stirred for 3 h at the same temperature. The mixture was diluted with saturated aq NaHCO<sub>3</sub> solution and DCM. The aqueous layer was separated and extracted with DCM. The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 30-50% EtOAc) to afford a yellow solid. The solid was dissolved with  $HCO_2H$  (64 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc and then basified with 30% aq Na<sub>2</sub>CO<sub>3</sub> solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (amino silica gel; hexane/EtOAc, gradient: 40-80% EtOAc) to afford 15 (7.03 g, 89% yield) as a pale yellow solid. <sup>1</sup>H-NMR (400MHz, CDCl<sub>3</sub>)  $\delta$ 1.66 (s, 3H), 4.26 (s, 2H), 4.74 (d, J = 47.7 Hz, 2H), 5.74 (dd, J = 5.1, 2.5 Hz, 1H), 7.06 (dd, J = 5.1, 2.5 11.2, 9.1 Hz, 1H), 7.69 (dd, J = 6.8, 2.8 Hz, 1H), 7.89–7.93 (m, 1H), 8.20 (dd, J = 8.1, 2.0 Hz, 1H), 8.42 (d, J = 8.1 Hz, 1H), 8.89 (d, J = 2.0 Hz, 1H), 9.84 (s, 1H). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>) § 31.66, 54.46, 80.40, 110.73, 112.40, 115.78, 116.74, 119.37, 119.90, 122.33, 133.14, 135.69, 141.25, 142.94, 148.21, 150.64, 152.25, 156.52, 159.99. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> calcd for  $C_{19}H_{16}F_2N_5O_2$ , 384.1267; found, 384.1261. [ $\alpha$ ]<sub>D</sub><sup>20</sup> + 34.7 (*c* 0.51, MeCN).

(*S*)-*N*-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl)-4-fluorophenyl)-5-cyano-3-methylpicolinamide (20). Compound 20 was prepared in a manner similar to that for 15 in 77% yield. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ 1.70 (s, 3H), 2.85 (s, 3H), 4.77 (d, *J* = 47.7 Hz, 2H), 5.80 (dd, *J* = 4.8, 2.6 Hz, 1H), 7.07 (dd, *J* = 11.3, 8.8 Hz, 1H), 7.71 (dd, *J* = 6.9, 2.7 Hz, 1H), 7.80–7.86 (m, 1H), 7.90–7.92 (m, 1H), 8.60–8.61 (m, 1H), 10.01 (br s, 1H). MS-ESI (*m/z*): 398 [M + H]<sup>+</sup>.

(*S*)-*N*-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl)-4-fluorophenyl)-3chloro-5-cyanopicolinamide (21). Compound 21 was prepared in a manner similar to that for 15 in 67% yield.<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ 1.65 (s, 3H), 4.73 (d, *J* = 47.7 Hz, 2H), 5.73 (dd, *J* = 5.1, 2.5 Hz, 1H), 7.06 (dd, *J* = 11.4, 8.9 Hz, 1H), 7.59 (dd, *J* = 6.8, 2.8 Hz, 1H), 7.91–7.95 (m, 1H), 8.16 (d, *J* = 2.0 Hz, 1H), 8.76 (d, *J* = 2.0 Hz, 1H), 9.67 (s, 1H). MS-ESI (*m/z*): 418 [M + H]<sup>+</sup>.

(*S*)-*N*-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl)-4-fluorophenyl)-5-cyano-3-methoxypicolinamide (22). Compound 22 was prepared in a manner similar to that for 15 in 83% yield. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ 1.80 (s, 3H), 4.03 (s, 3H), 4.79 (d, *J* = 47.2 Hz, 2H), 5.79 (dd, *J* = 4.3, 2.3 Hz, 1H), 7.07 (dd, *J* = 11.4, 8.9 Hz, 1H), 7.61 (d, *J* = 6.9, 2.7 Hz, 1H), 7.59–7.64 (m, 2H), 8.46–8.47 (m, 1H), 9.69 (s, 1H). MS-ESI (*m/z*): 414 [M + H]<sup>+</sup>.

## (S)-N-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4H-1,3-oxazin-4-yl)-4-fluorophenyl)-5-

**methoxypyrazine-2-carboxamide (23).** Compound **23** was prepared in a manner similar to that for **15** in 70% yield. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.65 (s, 3H), 4.07 (s, 3H), 4.73 (d, J = 47.7Hz, 2H), 5.73 (dd, J = 4.8, 2.3 Hz, 1H), 7.04 (dd, J = 11.4, 8.9 Hz, 1H), 7.65 (dd, J = 6.8, 2.8 Hz, 1H), 7.86–7.89 (m, 1H), 8.14 (s, 1H), 9.01 (s, 1H), 9.49 (s, 1H). MS-ESI (*m/z*): 390 [M + H]<sup>+</sup>.

# (S)-N-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4H-1,3-oxazin-4-yl)-4-fluorophenyl)-5-

(difluoromethyl)pyrazine-2-carboxamide (24). Compound 24 was prepared in a manner similar to that for 15 in 76% yield.<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.67 (s, 3H), 4.74 (d, *J* = 47.6 Hz, 2H), 5.75 (dd, *J* = 4.9, 2.6 Hz, 1H), 6.79 (t, *J* = 54.4 Hz, 1H), 7.07 (dd, *J* = 11.3, 8.8 Hz, 1H), 7.70 (dd, *J* = 6.9, 2.9 Hz, 1H), 7.86–7.92 (m, 1H), 8.91 (s, 1H), 9.51 (s, 1H), 9.62 (br s, 1H). MS-ESI (*m/z*): 410 [M + H]<sup>+</sup>.

(S)-N-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4H-1,3-oxazin-4-yl)-4-fluorophenyl)-5-

(fluoromethoxy)pyrazine-2-carboxamide (25). Compound 25 was prepared in a manner similar to that for 15 in 59% yield. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.66 (s, 3H), 4.74 (d, *J* = 47.7 Hz, 2H), 5.74 (dd, *J* = 4.8, 2.5 Hz, 1H), 6.15 (d, *J* = 51.0 Hz, 2H), 7.05 (dd, *J* = 11.4, 8.8 Hz, 1H), 7.67 (dd, *J* = 6.9, 2.8 Hz, 1H), 7.85–7.91 (m, 1H), 8.26–8.27 (m, 1H), 9.06 (s, 1H), 9.48 (br s, 1H). MS-ESI (*m/z*): 408. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>17</sub>F<sub>3</sub>N<sub>5</sub>O<sub>3</sub>, 408.1278; found, 408.1276.

# (S)-3-Amino-N-(3-(2-amino-6-(fluoromethyl)-4-methyl-4H-1,3-oxazin-4-yl)-4-

fluorophenyl)-5-(fluoromethoxy)pyrazine-2-carboxamide (26). Compound 26 was prepared in a manner similar to that for 15 in 95% yield. In this amidation reaction, the corresponding carboxylic acid lithium salt was used, and the reaction was conducted in DMF without DIEA. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.65 (s, 3H), 4.73 (d, *J* = 47.7 Hz, 2H), 5.74 (dd, *J* = 4.6, 2.4 Hz, 1H), 6.02 (d, *J* = 51.4 Hz, 2H), 7.01 (dd, *J* = 11.3, 8.8 Hz, 1H), 7.52 (s, 1H), 7.63–7.64 (m, 1H), 7.74–7.75 (m, 1H), 9.50 (s, 1H). HRMS-ESI (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>18</sub>F<sub>3</sub>N<sub>6</sub>O<sub>3</sub>, 423.1387; found, 423.1385.

# (S)-N-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4H-1,3-oxazin-4-yl)-4-fluorophenyl)-2-

(difluoromethyl)oxazole-4-carboxamide (27). Compound 27 was prepared in a manner similar to that for 15 in 64% yield. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ 1.65 (s, 3H), 4.74 (d, *J* = 47.6 Hz, 2H), 5.73 (dd, *J* = 4.7, 2.5 Hz, 1H), 6.69 (t, *J* = 52.4 Hz, 1H), 7.04 (dd, *J* = 11.2, 8.8 Hz, 1H), 7.62 (dd, *J* = 6.8, 2.8 Hz, 1H), 7.76–7.82 (m, 1H), 8.38 (s, 1H), 8.61 (br s, 1H). MS-ESI (*m/z*): 399 [M + H]<sup>+</sup>.

(*S*)-*N*-(3-(2-Amino-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-4-yl)-4-fluorophenyl)-2-(fluoromethyl)oxazole-4-carboxamide (28). Compound 28 was prepared in a manner similar to

that for **15** in 61% yield. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.73 (s, 3H), 4.76 (d, *J* = 47.4 Hz, 2H), 5.42 (d, *J* = 47.0 Hz, 2H), 5.76 (dd, *J* = 4.4, 2.4 Hz, 1H), 7.04 (dd, *J* = 11.3, 8.8 Hz, 1H), 7.64 (dd, *J* = 6.9, 2.7 Hz, 1H), 7.76–7.80 (m, 1H), 8.31–8.32 (m, 1H), 8.70 (br s, 1H). MS-ESI (*m/z*): 381 [M + H]<sup>+</sup>.

#### (*R*,*E*)-*N*-(1-(2-Fluoro-5-nitrophenyl)ethylidene)-2-methylpropane-2-sulfinamide (31). To a

solution of **30** (50.0 g, 273 mmol) and (*R*)-2-methylpropane-2-sulfinamide (36.4 g, 300 mmol) in THF (350 mL) was added Ti(OEt)<sub>4</sub> (106 g, 464 mmol) at room temperature, and the reaction mixture was stirred at 65 °C for 7 h. The mixture was allowed to cool to room temperature. To a solution of citric acid (166 g) in water/toluene (600 mL/660 mL) was added the reaction mixture at 10 °C. The mixture was stirred for 2 h and filtered through Celite. The aqueous layer was separated and extracted with toluene. The combined organic layers were evaporated, the resulting residue was treated with IPE (210 mL), and the mixture was stirred at 0 °C for 2 h. The resulting solid was collected on a Kiriyama funnel and washed with cooled IPE (100 mL) to afford **31** (50.6 g, 65% yield) as a white solid. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.34 (s, 9H), 2.81 (s, 3H), 7.29 (t, *J* = 9.6 Hz, 1H), 8.30–8.34 (m, 1H), 8.55–8.57 (m, 1H). MS-ESI (*m/z*): 287 [M + H]<sup>+</sup>.

#### (*R*,*E*)-*N*-(3-(1-((*tert*-Butylsulfinyl)imino)ethyl)-4-fluorophenyl)-2,2,2-trifluoroacetamide (32).

A suspension of **31** (48.0 g, 168 mmol), Fe (32.8 g, 587 mmol), and NH<sub>4</sub>Cl (40.4 g, 754 mmol) in toluene/H<sub>2</sub>O (480 mL/192 mL) was stirred at 80 °C for 6 h. The mixture was cooled to room temperature and diluted with EtOAc (150 mL) and water (200 mL). The insoluble materials were filtered off, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were evaporated. The residue was dissolved in THF (156 mL) and Et<sub>3</sub>N (58.1 mL, 419 mmol), and the mixture was cooled to –40 °C. To this mixture was added dropwise TFAA

(29.6 mL, 210 mmol) in THF (10 mL) at this temperature over 10 min, and the mixture was stirred at –25 °C for 30 min. The reaction was quenched with saturated aq NaHCO<sub>3</sub> solution (50 mL) at –25 °C, and the mixture was allowed to warm to room temperature and diluted with EtOAc (150 mL) and water (200 mL). The aqueous layer was separated and extracted with EtOAc. The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated. The residue was treated with IPE, and the mixture was stirred at room temperature. The resulting solid was collected on a Kiriyama funnel to afford **32** (46.0 g, 78% yield) as an orange solid. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.24 (s, 9H), 2.70 (s, 3H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.84–7.88 (m, 1H), 8.03–8.06 (m, 1H), 11.44 (br s, 1H). MS-ESI (*m/z*): 353 [M + H]<sup>+</sup>.

# N-(3-((S)-2-(((R)-tert-Butylsulfinyl)amino)pent-4-en-2-yl)-4-fluorophenyl)-2,2,2-

trifluoroacetamide (33). To a solution of 32 (10 g, 28.4 mmol) in THF (100 mL) was added allyl magnesium bromide (1 M in THF, 100 mL) at -78 °C for 1 h. The reaction was quenched with saturated aq NH<sub>4</sub>Cl solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0–30% EtOAc) to give 33 (4.5 g, 40% yield) as a yellow oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.21 (s, 9H), 1.68 (s, 3H), 2.79 (dd, *J* = 13.4, 7.3 Hz, 1H), 2.92 (dd, *J* = 13.4, 6.8 Hz, 1H), 4.16 (s, 1H), 5.10 (d, *J* = 9.6 Hz, 1H), 5.13 (d, *J* = 17.2 Hz, 1H), 5.51–5.65 (m, 1H), 6.95 (dd, *J* = 10.6, 10.1 Hz, 1H), 7.49–7.54 (m, 1H), 7.61–7.64 (m, 1H), 9.96 (s, 1H). MS-ESI (*m/z*): 395 [M + H]<sup>+</sup>.

*N*-(3-((*S*)-2-(((*R*)-*tert*-Butylsulfinyl)amino)-4-oxobutan-2-yl)-4-fluorophenyl)-2,2,2trifluoroacetamide (34). A solution of compound 33 (4.5 g, 11.4 mmol) in DCM (90 mL) was

cooled to -78 °C, and ozone gas was bubbled into the solution. When the color of the reaction solution changed to blue, the bubbling was stopped. Et<sub>3</sub>N (4.7 mL) was added to the soution, which was then stirred for an additional 1 h at -78 °C. The mixture was warmed to room temperature and diluted with EtOAc and water. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated to give **34** (4.0 g, 88% yield) as a yellow oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.11 (s, 9H), 1.79 (s, 3H), 3.18 (s, 2H), 5.49 (s, 1H), 7.20 (dd, *J* = 12.2, 8.8 Hz, 1H), 7.69 (ddd, *J* = 8.8, 4.0, 2.7 Hz, 1H), 7.84 (dd, *J* = 7.2, 2.7 Hz, 1H), 9.56 (s, 1H), 11.3 (s, 1H). MS-ESI (*m/z*): 397 [M + H]<sup>+</sup>.

(*S*)-*N*-(3-(2-Amino-4,4-dimethoxybutan-2-yl)-4-fluorophenyl)-2,2,2-trifluoroacetamide (35). To a solution of 34 (24.7 g, 62.3 mmol) in MeOH (250 mL) was added a solution of HCl (2 N in MeOH, 156 mL, 312 mmol). The mixture was stirred at room temperature for 22 h. The reaction mixture was evaporated, and then the residue was diluted with EtOAc. The mixture was poured into saturated aq NaHCO<sub>3</sub> solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 10–50% EtOAc) to give 35 (11.7 g, 56% yield) as a yellow oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.79 (s, 3H), 2.04 (dd, *J* = 14.4, 7.2 Hz, 1H), 2.34–2.41 (m, 1H), 3.20 (s, 3H), 3.21 (s, 3H), 4.08–4.16 (m, 1H), 7.06 (dd, *J* = 11.4, 8.7 Hz, 1H), 7.59–7.64 (m, 1H), 7.69 (dd, *J* = 7.2, 3.0 Hz, 1H), 8.04 (br s, 1H). MS-ESI (*m/z*): 443 [M + H]<sup>+</sup>.

(S)-N-(3-(2-(3,3-Diallylureido)-4,4-dimethoxybutan-2-yl)-4-fluorophenyl)-2,2,2trifluoroacetamide (36). To a solution of 35 (6.62 g, 19.6 mmol) and  $K_2CO_3$  (13.5 g, 98.0 mmol) in EtOAc (40 mL) and  $H_2O$  (26 mL) was added a solution of triphosgene (5.81 g, 19.6

mmol) in EtOAc (25 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h, and then a solution of diallylamine (5.70 g, 58.7 mmol) in EtOAc (13 mL) was added. The resulting mixture was stirred at 0 °C for 2 h and diluted with water. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 10–50% EtOAc) to give **36** (7.39 g, 82% yield) as a yellow oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.92–1.96 (m, 1H), 2.34 (dd, *J* = 14.7, 5.4 Hz, 1H), 3.33 (s, 3H), 3.34 (s, 3H), 3.86 (dd, *J* = 16.8, 4.8 Hz, 2H), 3.97 (dd, *J* = 16.8, 4.8 Hz, 2H), 4.34 (t, *J* = 5.4 Hz, 1H), 5.26 (m, 4H), 5.80–5.90 (m, 2H), 6.51 (s, 1H), 6.90 (dd, *J* = 11.7, 8.7 Hz, 1H), 7.38–7.42 (m, 1H), 6.90 (dd, *J* = 7.5, 2.7 Hz, 1H), 9.14 (s, 1H). MS-ESI (*m/z*): 462 [M + H]<sup>+</sup>.

# (S)-N-(3-(2-(Diallylamino)-4-methyl-4H-1,3-oxazin-4-yl)-4-fluorophenyl)-2,2,2-

trifluoroacetamide (37). To a solution of 36 (7.39 g, 16.0 mmol) in acetone (100 ml) was added a solution of  $H_2SO_4$  (1 N in water; 48 mL). The mixture was stirred at room temperature for 2 h. The mixture was diluted with EtOAc and water. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated to give a residue (6.36 g) as a yellow oil. The residue was dissolved in THF (50 mL). Burgess regent (7.30 g, 30.6 mmol) and pyridinium *p*toluenesulfonate (7.70 g, 30.6 mmol) were added to the solution, and the reaction mixture was refluxed for 7 h. After cooling to room temperature, the mixture was diluted with EtOAc and saturated aq NaHCO<sub>3</sub> solution. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0–20% EtOAc) to give **37** (1.45 g, 24%) as a colorless oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)

δ 1.60 (s, 3H), 3.85 (dd, J = 16.2, 5.7 Hz, 2H), 4.12–4.19 (m, 2H), 5.20–5.27 (m, 4H), 5.57 (dd, J = 6.0, 3.0 Hz, 1H), 5.83–5.95 (m, 2H), 6.38 (d, J = 6.0 Hz, 1H), 6.97–7.04 (m, 1H), 7.62–7.66 (m, 2H), 7.76 (br s, 1H). MS-ESI (m/z): 398 [M + H]<sup>+</sup>.

(*S*)-*N*,*N*-Diallyl-4-(5-amino-2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-2-amine (38). To a solution of **37** (624 mg, 1.57 mmol) in THF (2.0 mL), MeOH (2.5 mL), and H<sub>2</sub>O (2.5 mL) was added K<sub>2</sub>CO<sub>3</sub> (651 mg, 4.71 mmol). The mixture was stirred at 60 °C for 7 h. After being cooled to room temperature, the mixture was diluted with EtOAc and water. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated to give **38** (412 mg, 87% yield) as a colorless oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.55 (s, 3H), 3.49 (br s, 2H), 3.86 (dd, *J* = 15.7, 5.6 Hz, 2H), 4.05 (dd, *J* = 15.7, 5.6 Hz, 2H), 5.13–5.21 (m, 4H), 5.55 (dd, *J* = 6.1, 3.0 Hz, 1H), 5.81–5.92 (m, 2H), 6.34 (d, *J* = 6.1 Hz, 1H), 6.47–6.52 (m, 1H), 6.77 (dd, *J* = 11.7, 8.6 Hz, 1H), 7.03–7.06 (m, 1H). MS-ESI (*m*/z): 302 [M + H]<sup>+</sup>.

# (S)-5-Cyano-N-(3-(2-(diallylamino)-4-methyl-4H-1,3-oxazin-4-yl)-4-

**fluorophenyl)picolinamide (39).** To a solution of **38** (50.9 mg, 0.169 mmol), 5-cyanopocolinic acid monohydrate (33.7 mg, 0.203 mmol), 1-hydroxybenzotriazole hydrate (31.0 mg, 0.203 mmol), and DMAP (2.06 mg, 0.017 mmol) in DMF (1 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.9 mg, 0.203 mmol). The mixture was stirred at room temperature for 2 h. The mixture was diluted with EtOAc and saturated aq NaHCO<sub>3</sub> solution. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 0–30% EtOAc) to give **39** (66.9 mg, 92% yield) as a white solid. <sup>1</sup>H-NMR (400 MHz,

CDCl<sub>3</sub>)  $\delta$  1.60 (s, 3H), 3.85 (dd, J = 16.2, 5.6 Hz, 2H), 4.15 (dd, J = 16.2, 5.6 Hz, 2H), 5.20–5.26 (m, 4H), 5.58 (dd, J = 6.1, 3.0 Hz, 1H), 5.88–5.99 (m, 2H), 6.38 (d, J = 6.1 Hz, 1H), 7.03 (dd, J = 11.2, 8.6 Hz, 1H), 7.74–7.79 (m, 1H), 7.92–7.96 (m, 1H), 8.20 (dd, J = 8.1, 2.0 Hz, 1H), 8.43 (dd, J = 8.1, 1.0 Hz, 1H), 8.90 (d, J = 1.0 Hz, 1H), 9.79 (br s, 1H). MS-ESI (m/z): 432 [M + H]<sup>+</sup>.

#### (*R*,*E*)-*N*-(1-(2-Fluorophenyl)ethylidene)-2-methylpropane-2-sulfinamide (40). To a

suspension of 2-fluoroacetophenone (58.0 g, 420 mmol) and (*R*)-2-methylpropane-2-sulfinamide (66.2 g, 546 mmol) in toluene (290 mL) was added Ti(OEt)<sub>4</sub> (176 mL, 840 mmol) at room temperature, and the reaction mixture was stirred at 80 °C for 7 h. The reaction mixture was allowed to cool to room temperature and diluted with MeCN (1 L). To this mixture was added dropwise H<sub>2</sub>O (53 mL) at this temperature, and the mixture was stirred for 30 min. The resulting solid was filtered off, and the filtrate was evaporated to afford **40** (77.5 g, 77% yield) as an orange oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.32 (s, 9H), 2.77 (s, 3H), 7.06–7.13 (m, 1H), 7.15–7.20 (m, 1H), 7.39–7.45 (m, 1H), 7.65–7.70 (m, 1H). MS-ESI (*m/z*): 242 [M + H]<sup>+</sup>.

# (R)-N-((S)-2-(2-Fluorophenyl)pent-4-en-2-yl)-2-methylpropane-2-sulfinamide (41). To a

solution of allylmagnesium bromide (1 M in Et<sub>2</sub>O; 721 mL, 721 mmol) was added **40** (58.0 g, 240 mmol) in Et<sub>2</sub>O (58 mL) dropwise over 10 min at –60 °C. The mixture was allowed to warm to –20 °C and then stirred for 50 min. The misture was again cooled to –50 °C, and a solutin of NH<sub>4</sub>Cl (65 .0 g) in water (230 mL) was added dropwise to the mixture over 20 min. The aqueous layer was separated and then extracted with Et<sub>2</sub>O. The combined organic extracts were evaporated. The residue was treated with H<sub>2</sub>O (8.7 mL) then hexane (464 mL) at 0 °C, and the resulting suspension was stirred at 0 °C for 1 h. The resulting solid was collected on a glass filter and washed with hexane to afford **41** (55.3 g, 72% yield) as a white solid. Compound **41** was obtained as a dihydrate form. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.22 (s, 9H), 1.81 (s, 3H), 2.82 (dd,

*J* = 14.4, 7.5 Hz, 1H), 2.91 (dd, *J* = 14.4, 7.5 Hz, 1H), 3.99 (s, 1H), 5.07–5.11 (m, 1H), 5.12–5.19 (m, 1H), 5.46–5.56 (m, 1H), 7.00–7.07 (m, 1H), 7.09–7.14 (m, 1H), 7.26–7.31 (m, 1H), 7.35–7.41 (m, 1H). MS-ESI (*m/z*): 284 [M + H]<sup>+</sup>.

(*S*)-2-(2-Fluorophenyl)pent-4-en-2-amine (42). To a solution of 41 (47.8 g, 0.151 mmol) in MeOH (143 mL) was added HCl (4 N in EtOAc; 59.4 mL, 238 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. The mixture was poured into ice–water. It was then washed with water and basified with NaOH solution (2N in water, 135 mL). The aqueous layer was separated and extracted with Et<sub>2</sub>O. The combined organic extracts were evaporated to afford 42 (30.0 g, 106% yield) as a colorless oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.52 (s, 3H), 2.48 (dd, *J* = 13.5, 7.8 Hz, 1H), 2.74 (dd, *J* = 13.5, 7.8 Hz, 1H), 4.99–5.09 (m, 2H), 5.47–5.61 (m, 1H), 6.98–7.05 (m, 1H), 7.06–7.12 (m, 1H), 7.17–7.22 (m, 1H), 7.39–7.45 (m, 1H). MS-ESI (*m/z*): 180 [M + H]<sup>+</sup>.

#### (S)-1,1-Bis(2,4-dimethoxybenzyl)-3-(2-(2-fluorophenyl)pent-4-en-2-yl)urea (43). To a

mixture of **42** (28.1 g, 148 mmol) and K<sub>2</sub>CO<sub>3</sub> (2 M in H<sub>2</sub>O; 82 mL, 164 mmol) in EtOAc (420 mL) and H<sub>2</sub>O (82 mL) was added 4-nitrophenyl chloroformate (31.4 g, 156 mmol) at 0 °C, and the suspension was stirred at the same temperature for 15 min. Bis(2,4-dimethoxybenzyl)amine (49.5 g, 156 mmol) was added portionwise at 0 °C, and the mixture was warmed to room temperature and then stirred for 4 h. The reaction mixture was diluted with water and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were washed with aq K<sub>2</sub>CO<sub>3</sub> solution and water, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was crystallized from EtOAc, and the resulting solid was collected on a glass filter to afford **43** (63.2 g, 82% yield) as a colorless solid. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.76 (s, 3H), 2.52 (dd, *J* = 13.2, 7.6 Hz, 1H), 2.89 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.76 (s, 6H), 3.81 (s, 6H), 4.37-

4.46 (m, 4H), 4.88–4.90 (m, 1H), 4.90–4.95 (m, 1H), 5.35 (s, 1H), 5.42–5.53 (m, 1H), 6.43–6.47 (m, 4H), 6.97 (ddd, *J* = 12.8, 8.4, 1.2 Hz, 1H), 7.05 (td, *J* = 7.6, 1.6 Hz, 1H), 7.14–7.20 (m, 3H), 7.27 (td, *J* = 8.4, 1.6 Hz, 1H). MS-ESI (*m/z*): 523 [M + H]<sup>+</sup>.

#### (S)-1,1-Bis(2,4-dimethoxybenzyl)-3-(2-(2-fluorophenyl)-5-hydroxy-4-oxopentan-2-yl)urea

(44). To a mixture of 43 (2.97 g, 5.68 mmol) in acetone (30 mL) and AcOH (2 M in H<sub>2</sub>O; 9.0 mL) was added dropwise a solution of KMnO<sub>4</sub> (1.62 g, 10.2 mmol) and AcOH (1 M in H<sub>2</sub>O; 15 mL) over 15 min on a water bath, and the reaction mixture was stirred at room temperature for 30 min. The reaction was quenched with 10% aq NaHSO<sub>3</sub> solution (10 mL) and then evaporated acetone. The resulting mixture was extracted with EtOAc, and the combined organic layers were washed with 10% aq Na<sub>2</sub>CO<sub>3</sub> solution and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 50–80% EtOAc) to give 44 (2.13 g, 68% yield) as a while amorphous. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.77 (s, 3H), 3.07–3.11 (m, 1H), 3.76–3.81 (m, 1H), 3.77 (s, 6H), 3.81 (s, 6H), 3.90–3.93 (m, 1H), 4.07–4.09 (m, 1H), 4.36–4.48 (m, 4H), 5.87 (s, 1H), 6.43–6.49 (m, 4H), 6.97–7.06 (m, 2H), 7.13–7.21(m, 4H). MS-ESI (*m*/*z*): 555 [M + H]<sup>+</sup>.

# (*S*)-(2-(Bis(2,4-dimethoxybenzyl)amino)-4-(2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-6yl)methyl methanesulfonate (45). To a solution of 44 (2.13 g, 3.84 mmol) and Et<sub>3</sub>N (799 μL, 5.76 mmol) in DCM (20 mL) was added methanesulfonic anhydride (736 mg, 4.22 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. The reaction mixture was diluted with saturated aq NaHCO<sub>3</sub> solution. The aqueous layer was separated and extracted with DCM. The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was diluted with THF (25 mL). To the mixture was added Burgess reagent (1.83 g, 7.68 mmol), and the reaction mixture was stirred at room temperature for 2 h.

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The mixture was diluted with water, and the aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with water and brain, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 30-50% EtOAc) to give **45** (944 mg, 40% yield) as a while amorphous. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.61 (s, 3H), 2.70 (s, 3H), 3.75 (s, 6H), 3.80 (s, 6H), 4.46 (d, J = 16.0 Hz, 2H), 4.55 (s, 2H), 4.66 (d, J = 16.0 Hz, 2H), 5.82 (d, J = 3.2 Hz, 1H), 6.43–6.46 (m, 4H), 6.93–7.00 (m, 2H), 7.13–7.18 (m, 3H), 7.46–7.51 (m, 1H). MS-ESI (*m/z*): 615 [M + H]<sup>+</sup>.

# (S)-N,N-Bis(2,4-dimethoxybenzyl)-4-(2-fluorophenyl)-6-(iodomethyl)-4-methyl-4H-1,3-

oxazin-2-amine (46). To a solution of 45 (20.5 g, 33.4 mmol) in acetone (200 mL) was added NaI (10.0 g, 66.7 mmol) at room temperature. After stirring at the same temperature for 1 h, the mixture was filtered through Celite, and the filtrate was evaporated. The residue was diluted with EtOAc and water, and the aqueous layer was separated and then extracted with EtOAc. The combined organic layers were washed with 10% aq Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 20–40% EtOAc) to give **46** (20.5 g, 95% yield) as a pale yellow amorphous. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.55 (s, 3H), 3.72–3.73 (m, 2H), 3.75 (s, 6H), 3.81 (s, 6H), 4.48 (d, *J* = 16.0 Hz, 2H), 4.67 (d, *J* = 15.6 Hz, 2H), 5.72 (d, *J* = 3.2 Hz, 1H), 6.43–6.47 (m, 4H), 6.93–6.99 (m, 2H), 7.12–7.18 (m, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 7.51 (td, *J* = 8.4, 2.0 Hz, 1H). MS-ESI (*m/z*): 647 [M + H]<sup>+</sup>.

# (*S*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-6-(fluoromethyl)-4-(2-fluorophenyl)-4-methyl-4*H*-1,3oxazin-2-amine (47). To a solution of 46 (20.5 g, 31.7 mmol) in MeCN (120 mL) was added AgF (10.1 g, 79.6 mmol) at room temperature. After being stirred at the same temperature for 16

h, the mixture was filtered through Celite, and the filtrate was evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 10–30% EtOAc) to give **47** (16.8 g, 98% yield) as a colorless amorphous. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.60 (s, 3H), 3.76 (s, 6H), 3.81 (s, 6H), 4.46 (d, *J* = 15.6 Hz, 2H), 4.65 (d, *J* = 16.0 Hz, 2H), 4.67 (d, *J* = 47.6 Hz, 2H), 5.76–5.78 (m, 1H), 6.44–6.46 (m, 4H), 6.93–6.99 (m, 2H), 7.12–7.19 (m, 3H), 7.48–7.52 (m, 1H). MS-ESI (*m/z*): 539 [M + H]<sup>+</sup>.

(*S*)-6-(Fluoromethyl)-4-(2-fluorophenyl)-4-methyl-4*H*-1,3-oxazin-2-amine (48). A mixture of 47 (16.8 g, 31.1 mmol) and anisole (23.8 mL, 218 mmol) in TFA (84 mL) was stirred at 80 °C for 17 h. The reaction mixture was warmed to room temperature and evaporated. The residue was diluted with EtOAc and 10% aq Na<sub>2</sub>CO<sub>3</sub> solution. The aqueous layer was separated and extracted with EtOAc. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by column chromatography (silica gel; hexane/EtOAc, gradient: 50–80% EtOAc) to give 48 (7.64 g, 103% yield) as a brown oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.65 (s, 3H), 4.20 (br s, 2H), 4.72 (d, *J* = 48.0 Hz, 2H), 5.70 (dd, *J* = 4.8, 2.4 Hz, 2H), 7.01 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.12 (td, *J* = 7.6, 1.2 Hz, 1H), 7.19–7.26 (m, 1H), 7.54 (td, *J* = 8.4, 2.0 Hz, 1H). MS-ESI (*m/z*): 239 [M + H]<sup>+</sup>.

#### (S)-4-(2-Fluoro-5-nitrophenyl)-6-(fluoromethyl)-4-methyl-4H-1,3-oxazin-2-amine (49).

Compound **48** (7.41 g, 31.1 mmol) was dissolved in TFA (60 mL). The reaction mixture was cooled to -20 °C, and H<sub>2</sub>SO<sub>4</sub> (15 mL) was added. To the mixture was added dropwise HNO<sub>3</sub> (1.42 g/mL; 2.09 mL, 46.7 mmol) at -20 °C over 10 min. The reaction mixture was poured into ice water and basified with 20% aq Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was extracted with EtOAc, and the combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered, and

evaporated. The residue was purified by column chromatography (amino silica gel; hexane/EtOAc, gradient: 30–50% EtOAc) to give **49** (8.41 g, 94% yield) as a pale yellow amorphous. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.65 (s, 3H), 4.30 (br s, 2H), 4.74 (d, *J* = 47.2 Hz, 2H), 5.69 (dd, *J* = 4.4, 2.8 Hz, 2H), 7.16 (dd, *J* = 10.4, 8.8 Hz, 1H), 8.12–8.16 (m, 1H), 8.53 (dd, *J* = 6.8, 2.8 Hz, 1H). MS-ESI (*m/z*): 284 [M + H]<sup>+</sup>.

*tert*-Butyl *N*-[(*tert*-butoxy)carbonyl]-*N*-[(4*S*)-4-(2-fluoro-5-nitrophenyl)-6-(fluoromethyl)-4methyl-4*H*-1,3-oxazin-2-yl]carbamate (50). To a solution of 49 (8.41 g, 29.7 mmol) and DMAP (363 mg, 2.97 mmol) in THF (80 mL) was added Boc<sub>2</sub>O (14.5 mL, 62.4 mmol) at 0 °C, and the reaction mixture was warmed to room temperature and stirred for 2 h. The mixture was diluted with 15% aq citric acid solution and EtOAc. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with water, saturated aq NaHCO<sub>3</sub> solution and brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was crystallized from Et<sub>2</sub>O to afford 50 (12.9 g, 90% yield) as a pale yellow solid. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  4.76 (dd, *J* = 47.2, 3.6 Hz, 2H), 5.63 (dd, *J* = 4.0, 2.8 Hz, 1H), 7.22 (dd, *J* = 10.4, 9.2 Hz, 1H), 8.17–8.21 (m, 1H), 8.52 (dd, *J* = 6.8, 2.8 Hz, 1H). MS-ESI (*m*/z): 484 [M + H]<sup>+</sup>.

*tert*-Butyl *N*-[(4*S*)-4-(5-amino-2-fluorophenyl)-6-(fluoromethyl)-4-methyl-4*H*-1,3-oxazin-2yl]-*N*-[(*tert*-butoxy)carbonyl]carbamate (51). A suspension of 50 (12.9 g, 26.7 mmol), Fe (11.9 g, 213 mmol), and NH<sub>4</sub>Cl (17.1 g, 320 mmol) in EtOH/THF/H<sub>2</sub>O (100 mL/50 mL/50 mL) was stirred at 60 °C for 2 h. The reaction mixture was warmed to room temperature and filtered through Celite. The Celite was washed with EtOAc and water. The aqueous layer was separated and extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was crystallized from EtOAc/hexane to afford 51

(10.3 g, 85% yield) as a yellow solid. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.51 (s, 18H), 1.69 (s, 3H), 3.54 (s, 2H), 4.74 (dd, J = 47.2, 2.0 Hz, 2H), 5.66 (dd, J = 4.8, 1.6 Hz, 1H), 6.49–6.53 (m, 1H), 6.80–6.87 (m, 2H). MS-ESI (m/z): 454 [M + H]<sup>+</sup>.

# **ASSOCIATED CONTENT**

# **Supporting Information**

Figure S1, experimental procedures and characterization data for compounds **12–14**, **16–18**, and the carboxylic acids for **25** and **26**, crystallographic information, methods for biological, DMPK, physicochemical, and toxicological assays (PDF). SMILE strings, BACE1 IC<sub>50</sub>, cellular A $\beta$  IC<sub>50</sub>, P-gp ER, hERG % inhibition at 5  $\mu$ M, LogD, p $K_a$ , % remaining in HLM, and % remaining in RLM (CSV). This material is available free of charge via the Internet at http://pubs.acs.org.

## **Accession Codes**

The PDB accession code for the X-ray structure of 15 bound to human BACE1 is 5YGY.

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#### **Author Contributions**

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# Notes

The authors declare no competing financial interest.

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# **ABBREVIATIONS**

AD, Alzheimer's disease; Aβ, amiloid β; BACE1, β-site APP cleaving enzyme; *B/P*, brain-toplasma; CE, capillary electrophoresis; CNS, central nervous system; DMA, *N*,*N*dimethylacetamide; DMAP, *N*,*N*-dimethyl-4-aminopyridine; DMP, Dess-Martin periodinane; DMPU, *N*,*N'*-dimethylpropyleneurea; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; ER, efflux ratio; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5*b*]pyridinium 3-oxid hexafluorophosphate; HPBCD, 2-hydroxypropyl-β-cyclodextrin; HLM, human liver microsomes; HOBt, 1,2,3-benzotriazol-1-ol monohydrate; HTS, high-throughput screening; IPE, isopropyl ether; LLC-PK, pig-kidney derived epithelial; MC, methylcellulose; MDCK, Mardin-Darby canine kidney; MDR, multidrug resistance; NOAEL, no observable adverse effect level; P-gp, P-glycoprotein; PPTS, pyridinium *p*-toluenesulfonate; RLM, rat liver microsomes; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride.

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# **Table of Contents Graphic and Synopsis**



