Aminomethyl-Derived Beta Secretase (BACE1) Inhibitors: Engaging Gly230 without an Anilide Functionality

Christopher R. Butler,* Kevin Ogilvie,§ Luis Martinez-Alsina,§ Gabriela Barreiro,† Elizabeth M. Beck,‡ Charles E. Nolan,§ Kevin Atchison,§ Eric Benvenuti,⊥ Leanne Buzon,§ Shawn Doran,⊥ Cathleen Gonzales,⊥ Christopher J. Helal,§ Xinjun Hou,† Mei-Hui Hsu,§ Eric F. Johnson,⊥ Kimberly Lapham,⊥ Lorraine Lanyon,⊥ Kevin Parris,∥ Brian T. O’Neill,§ David Riddell,‡ Ashley Robshaw,‡ Felix Vajdos,∥ and Michael A. Brodney†

†Neuroscience & Pain Medicinal Chemistry, ‡Neuroscience & Pain Research Unit, Pfizer Worldwide Research and Development, 610 Main Street, Cambridge, Massachusetts 02139, United States
§Neuroscience & Pain Medicinal Chemistry, ∥Center of Chemistry Innovation and Excellence, ⊥Pharmacokinetics, Dynamics, and Metabolism, Pfizer Worldwide Research and Development, 445 Eastern Point Road, Groton, Connecticut 06340, United States
#Molecular and Experimental Medicine, The Scripps Research Institute, 10550 Torrey Pines Road, La Jolla, California 92037, United States

Supporting Information

ABSTRACT: A growing subset of β-secretase (BACE1) inhibitors for the treatment of Alzheimer’s disease (AD) utilizes an anilide chemotype that engages a key residue (Gly230) in the BACE1 binding site. Although the anilide moiety affords excellent potency, it simultaneously introduces a third hydrogen bond donor that limits brain availability and provides a potential metabolic site leading to the formation of an aniline, a structural motif of prospective safety concern. We report herein an alternative aminomethyl linker that delivers similar potency and improved brain penetration relative to the amide moiety. Optimization of this series identified analogues with an excellent balance of ADME properties and potency; however, potential drug−drug interactions (DDI) were predicted based on CYP 2D6 affinities. Generation and analysis of key BACE1 and CYP 2D6 crystal structures identified strategies to obviate the DDI liability, leading to compound 16, which exhibits robust in vivo efficacy as a BACE1 inhibitor.

INTRODUCTION

Alzheimer’s disease (AD), a neurological disorder that imparts a slow progression of cognitive decline, dementia, and ultimately death, has yet to yield to a significant enhancement in treatment or prevention. Disease progression is marked by the deposition of amyloid β (Aβ)-derived plaques in the hippocampal and cortical regions of the brain. The amyloid hypothesis proposes that increased Aβ production or its decreased clearance is responsible for the molecular cascade that eventually leads to neurodegeneration and AD. Aβ production is initiated by the prolytic cleavage of amyloid precursor protein (APP) by β-site APP cleaving enzyme (BACE1) within the endosome to afford a soluble N-terminal ectodomain of APP (sAPPβ) and the C-terminal fragment C99. The membrane-bound C99 is then cleaved by γ-secretase to release Aβ, including Aβ40 and Aβ42 isoforms.

Recently, an APP “loss of function” mutation, with protective effects against AD, has been reported to be cleared more slowly by BACE1. Modulation of the Aβ cascade via safe and effective inhibition of BACE1 has remained a target of great interest for a number of years. Considering the chronic dosing regimen required for a successful AD treatment, an exquisitely selective and safe profile for a BACE1 inhibitor is paramount. Of particular concern for this target is inhibition of hERG, as well as related aspartyl proteases including cathepsin D (CatD), which has confounded early generations of BACE1 inhibitors. The hERG-mediated cardiovascular liability is traditionally avoided by eliminating basic amine functionality and lowering lipophilicity. This is
challenging for BACE1, as the active site is most efficiently engaged through utilization of such an amine, thus requiring alternate mitigation strategies. Additionally, the binding sites of CatD and BACE1 have high sequence similarity, and therefore differentiation requires exploitation of subtle architectural variances in order to maintain affinity for BACE1 while avoiding CatD inhibition. Compounds that fail to achieve sufficient selectivity over CatD carry a liability for ocular toxicity due to the resulting accumulation of fluorescent material in the retinal pigment epithelium (RPE) layer.9

The physiological relevance of BACE2 has emerged in recent years, first as an enzyme involved in pigmentation processing, specifically acting on PMEL17 in the periphery.11 Improper functioning of BACE2 is believed to result in hypopigmentation.12 BACE2 is also expressed in the pancreas and plays a role in glucose homeostasis. To our knowledge, there are limited examples of BACE1 inhibitors possessing significant selectivity over BACE2. Compounds that lack this selectivity window and exhibit impaired access to the brain will therefore inherently suffer from significant inhibition of BACE2. In summary, agents developed for chronic BACE1 inhibition should be designed to minimize activity against related proteases such as CatD and BACE2.

The amidine-containing BACE1 inhibitors, reported by a number of groups, provide a suitable scaffold to systematically address the CatD and hERG liabilities.13 A number of these inhibitors, such as MK-8931 (1), have recently entered clinical studies; two of them are shown in Figure 1.14 A common construct within this class is an amide moiety connecting two aromatic rings that ultimately occupy the S1/S3 pockets when bound in the BACE1 active site. The incorporation of this moiety generally confers potent inhibition of BACE1 in addition to exquisite selectivity over CatD. Unfortunately, these merits are generally offset by increased P-gp-mediated efflux, resulting in decreased brain penetration. There is a correlation between the presence of a third hydrogen bond (HBD) and an increased likelihood of efflux transporter liabilities.15 Poor brain penetration inherently increases the body burden required to achieve the desired brain concentrations, and thus further exacerbates any issues arising from less than exquisite aspartyl protease selectivity. Moreover, recent reports have shown that there are relevant peripheral substrates for BACE1 in addition to the targeted central APP processing.16

Inhibitors bearing the P1/P3 amide motif not only exhibit higher efflux transporter liability but also contain a metabolic soft spot associated with amidase activity, which in this case would reveal anilines upon amide cleavage.17 In addition to this potential metabolic liability, anilines are themselves a structural alert, known to be a culprit for downstream toxicity associated either with oxidation of the electron-rich aryl ring and subsequent trapping with ambient nucleophiles or oxidation of the nitrogen itself to the N-oxide.18

**DESIGN CRITERIA**

Despite a significant and sustained effort, the identification of a potent, safe, selective BACE1 inhibitor with a balanced ADME profile, including good brain penetration, remains challenging. Our design criteria were therefore to identify an orally efficacious BACE1 inhibitor that (a) demonstrates excellent selectivity (>100×) over hERG, CatD and the related aspartyl proteases, and (b) maintains good brain penetration (Cbrain/Cplasma > 0.5 as measured in rodents) without the use of an anilide functionality.

We therefore sought to identify an amide replacement that provided the opportunity for similar efficiency gains while avoiding the potential for toxicity imparted by the buried aniline moiety. The recent BACE1 crystal structure of 2, published by Roche, provides a structural rationale for the high inhibitory efficiency of P1/P3 amides (Figure 2).19 The amide

![Figure 1. Selected literature BACE1 inhibitors.](image)

![Figure 2. Interaction between Gly230 and 2 (PDB: 3ZMG, add 61 to residue numbers to match 3ZMG’s number).](image)
ability of the amine with the resultant ADME characteristics of these compounds.

RESULTS AND DISCUSSION

As it was not clear what structural attributes would be required to fill the S3 pocket in this new series, parallel (library) synthesis was employed to broadly evaluate structure–activity relationships for BACE1 inhibition. Compounds 6a–u were prepared by the three-step protocol illustrated in Scheme 1. Formylation of the previously described bromide 3 provided aldehyde 4, which could be converted to the corresponding protected amidines 5 via a reductive amination with the requisite amines. Removal of the amidine protecting group using standard conditions provided the analogues of interest.

Analogues were evaluated in a panel of BACE1, BACE2, and CatD enzymatic assays (cell-free assay, CFA), as well as a whole-cell assay (WCA) reporting changes in soluble APP (sAPPβ) protein concentrations, indicative of APP processing by BACE1. The data from this assay panel, as well as key ADME parameters, for 6a–6k are provided in Table 1. The direct replacement of the amide linker with an aminomethylene spacer, as exemplified by 6a, yields weak activity at BACE1 (CFA IC_{50} = 73.5 μM), in stark contrast to the low nanomolar potency observed for many of the corresponding amides reported in the literature. In addition, the significant ADME challenges for this series are well illustrated by 6a, which exhibits a high MDR efflux ratio (Er) and significant clearance in human liver microsomes (HLM). Analogues 6b–d, which contain small alkyl substituents, show a modest improvement in BACE1 CFA potency, in concert with a dramatic increase in WCA potency (∼1000-fold relative to the CFA) and excellent selectivity over CatD. The improvement is most marked when considering ligand and lipophilic efficiencies (LE, LipE) for these low molecular weight, polar, dibasic amine analogues. These compounds have significantly reduced clearance relative to 6a, as measured by HLM, although they still exhibit significant P-gp transporter liability. Tertiary amines 6d–e are significantly less active in the BACE1 CFA but still exhibit good potency in the WCA, albeit an overall decrease in LipE, relative to the secondary amines. Ether-containing substituents are also well tolerated (6e,f), showing similar potency in the WCA, low microsomal clearance but with modest to high efflux ratios. Introduction of a branched methyl group (6c vs 6b and 6h vs 6f) offers an enhancement in potency (WCA) and a modest improvement in selectivity over BACE2. Tying this branching back into a 1,1,1-bicyclopentane (6i) is tolerated from a potency perspective but negatively impacts the clearance, potentially due to the increased overall lipophilicity.

Reduction in the pK_a of the benzylic amine center (“pK_a2”) was attractive due to the potential to simultaneously impact P-gp efflux and hERG liabilities. Indeed, addition of an electron-withdrawing trifluoromethyl group onto the amine significantly improves both potency and ADME balance. Although the tertiary amine (6j) is much less potent in the WCA, the secondary amine (6k) retains good WCA potency and comparable LipE to the more basic analogues and exhibits enhanced BACE1 CFA potency and improved selectivity over BACE2 (5.8x vs 3.2x for 6f). Although both CF3 analogues have significantly improved MDR-based efflux ratios (1.7 and 1.2), the secondary amine 6k shows greater metabolic stability (HLM CL_{int} = 12.0 mL/min/kg) than the methylated version 6j (HLM = 29.4 mL/min/kg).

It was clear that the additional basic amine exerts a profound effect on whole-cell potency, reinforced by comparison to the previously described monocyclic analogue devoid of a P3 group (compound 6 in ref 21, BACE1 CFA IC_{50} = 36 μM, WCA IC_{50} = 636 nM). The low molecular weight and dibasic nature of

Scheme 1

Reagents and conditions: (a) MeLi, Et_2O, n-BuLi, then DMF, −78 °C, 92%; (b) amine, Na(OAc),BH, DCE, rt, 22–97%; (c) HCl, dioxane, rt, 19–90%; (d) benzoic anhydride, TEA, THF/MeOH (2:1), rt, 86%; (e) N_2H_4·H_2O, EtOH, rt, 31%. P = Boc or benzoyl.
the small alkyl exemplars result in highly ligand- and lipophilic efficient compounds in the whole-cell assay. While a similar rank order trend is observed for the BACE1 CFA, there is a 20−7750-fold disconnect observed between the two assay formats. This disconnect can be rationalized by the presence of the second basic center increasing the propensity for accumulation into the endosome, an acidic intracellular compartment where BACE1 is thought to be localized,3 in the WCA format. As expected, decreasing the basicity of the amine by the installation of an electron-withdrawing group significantly compresses the CFA/WCA disconnect.

It was recognized from the outset that the diversion from an amide moiety would likely result in a very different BACE1 substituent-derived SAR relative to the amides, as well as presenting distinct challenges to achieving a balanced ADME profile. A second round of amine optimization (Table 2) explored utilization of an electron-withdrawing group to balance ADME properties as well as incorporation of branched amines to further enhance potency. In an effort to more clearly define the impact of the EWGs on ADME balance, we measured the pK₂ for the amidine ("pK₂") and benzylic amine ("pK₂") for each of the analogues. Removal of one of the fluorines (6l) increases pK₂ relative to analogue 6k, therefore increasing MDR Er (1.2 vs 3.1). Introduction of two geminal methyl groups provides a significant improvement in BACE1 CFA potency (6m vs 6k), albeit with a corresponding increase in MDR Er and a decrease in BACE2 selectivity (2×). Removal of one of the methyl groups (6n) decreases potency both in BACE1 CFA (1.9 μM) and WCA (64 nM) but improves efflux and BACE2 selectivity while reducing CYP 2D6 inhibition. Increasing the distance between the amine and trifluoromethyl groups (6o) increases pK₂ and diminishes potency in both assay formats. In an effort to capitalize on the potency increase associated with increased substitution, cyclopropylamine-containing analogues (6p−q) were prepared. The cyclopropane unit is well tolerated, as exhibited by the excellent WCA potency, but suffers from a significant degradation in BACE1 CFA potency and MDR Er. The addition of a

Table 1. In Vitro Data for 6a–k

| Cpd | R | BACE1 CFA IC₅₀ (μM)ᵃ | BACE2 CFA IC₅₀ (μM)ᵇ | CatD CFA IC₅₀ (μM)ᶜ | BACE1 WCA IC₅₀ (μM)ᵈ | MDR Er² | LogD | HLM² (mL/mg) | WCA LE/LipE |
|-----|---|----------------------|----------------------|----------------------|----------------------|--------|------|-------------|------------|
| 6a  |   | 73.5                 | >100                 | 1.35                 | 8.8                  | 2.4    | 199  | 0.34/5.2   |            |
| 6b  | Me | 13.9                 | 75.7                 | >100                 | 0.008 (1,737x)       | 2.0    | <1.5 | <8/9.9     |            |
| 6c  | Me | 15.5                 | >94                  | >100                 | 0.002 (7,750x)       | ND     | <1.5 | ND/10.2    |            |
| 6d  | Me | 50.9                 | >100                 | >100                 | 0.022 (2,313x)       | ND     | 0.3  | <8/7.6     |            |
| 6e  | MeO | 40.8                 | >100                 | >100                 | 0.038 (1,073x)       | 7.9    | 0.4  | 17.4/4.4/7.3 |            |
| 6f  | MeO | 8.20                 | 26.8                 | >100                 | 0.006 (1,366x)       | 3.9    | -0.3 | <8/8.2     |            |
| 6g  | MeO₂ | 35.1                 | 62.9                 | >100                 | 0.053 (1,186x)       | 9.0    | -0.5 | <8/4.5/8.1 |            |
| 6h  | Me | 4.18                 | 19.4                 | >100                 | 0.004 (1,045x)       | 6.8    | -0.3 | <8/5.2/9.0 |            |
| 6i  | F₂C | 2.10                 | 17.5                 | 95.3                 | 0.004 (525x)         | 5.2    | 1.0  | 14.8/5.2/7.7 |            |
| 6j  | F₂C | 20.2                 | <84                  | >100                 | 1.013 (19.9x)        | 1.7    | 1.2  | 29.4/3.6/5.1 |            |
| 6k  | F₂C | 1.31                 | 7.6                  | >100                 | 0.060 (21.8x)        | 1.2    | 1.0  | 12.0/4.5/6.7 |            |

ᵃIC₅₀ values obtained from BACE1 CFA.ᵇIC₅₀ values obtained from BACE2 CFA.ᶜIC₅₀ values obtained from CatD CFA.ᵈIC₅₀ values obtained from BACE1 WCA; value in parentheses is the BACE1 CFA/WCA ratio.²Ratio from the MS-based quantification of apical/basal and basal/apical transfer rates of a test compound at 2 μM across contiguous monolayers from MDR1-transfected MDCK cells.²Hepatic clearance predicted from in vitro human microsomal stability study.
diﬂuoromethyl group (6r) improves the BACE1 CFA potency and reduces the increase in MDR Er but simultaneously introduces a metabolic liability (HLM = 22 mL/min/kg). Replacement with a nitrile (6s) enhances BACE2 selectivity, but the MDR Er increases signiﬁcantly (MDR Er = 6.0). Use of an oxetane (6t) in place of the cyclopropane improves WCA potency and decreases log D but results in an elevated MDR Er ratio (MDR Er = 3.5) nearly equivalent to that of the parent cyclopropylamine 6p. In contrast, the CF3-cyclopropyl derivative 6u maintains good potency in both assay formats, exhibits a minimal eﬄux ratio and low clearance, and maintains excellent selectivity over CatD (470×) and modest selectivity over BACE2 (5.9×).

Although the decrease in pK_a imparted by the introduction of the cyclopropyl group in 6u (2.9 vs 3.8 for 6k) could contribute to the improved BACE1 CFA potency, it is not sufﬁcient to explain the 20-fold potency increase observed. To better rationalize this improvement, a co-crystal structure of compound 6u in BACE1 was obtained (PDB: ST1U). As shown in Figure 4, the thioamide and diﬂuorophenyl ring systems are oriented in a similar overall fashion to previously described BACE1 structures, with the diﬂuorophenyl occupying the S1 pocket as expected. As predicted by our initial design hypothesis, the benzylic amine substituent adopts an orientation orthogonal to the plane of the diﬂuorophenyl P1 group to optimally engage the carbonyl of Gly230. Interestingly, the triﬂuoromethyl group orients toward and ﬁlls the entrance to the S3 pocket, while the cyclopropyl substituent ﬁlls a small, lipophilic pocket at the back of the interface between the S1 and S3 pockets. Arguably, the signiﬁcant

---

**Table 2. In Vitro Data for Compounds 6k–6u**

| Cpd  | R          | BACE1 CFA IC50 (μM)a | BACE2 CFA IC50 (μM)b | CatD CFA IC50 (μM)c | BACE1 WCA IC50 (μM)d | MDR Er e | LogD | HLM (mL/ min/ kg)f | CYP 2D6% inhibition g | pK_a,2 h | HERG (nM)i |
|------|------------|----------------------|----------------------|---------------------|----------------------|---------|------|-----------------|------------------------|----------|------------|
| 6k   | F3C        | 1.31                 | 7.60                 | >100                | 0.060                | 1.2     | 1.0  | 12.0           | 25%                    | 8.9/5.2  | 6.1        |
| 6l   | HF2C       | 5.89                 | 27.3                 | >100                | 0.058                | 3.1     | 0.3  | 13.0           | 21%                    | 8.9/4.2  | 14.7       |
| 6m   | Me Me      | 0.49                 | 1.02                 | >100                | 0.031                | 3.6     | 1.5  | <8.0           | 81%                    | 8.9/3.4  | 11.2       |
| 6n   | Me Me      | 1.97                 | 7.13                 | >100                | 0.064                | 2.2     | 0.6  | 11.9           | 45%                    | 8.8/3.4  | 13.9       |
| 6o   | F3C N     | 54.7                 | >100                 | >100                | 0.400                | 6.1     | 0.5  | 9.3            | 39%                    | 8.9/6.0  | 20.4       |
| 6p   | N          | 20.6                 | >100                 | >100                | 0.030                | 3.4     | 0.5  | <8.0           | 14%                    | 9.0/6.2  | 49.2       |
| 6q   | N          | 15.4                 | 35.9                 | >100                | 0.014                | 6.9     | 0.1  | <8             | 12%                    | 9.3/6.3  | 33.0       |
| 6r   | HF2C       | 0.335                | 1.01                 | 65.5                | 0.012                | 2.5     | 0.7  | 22.2           | 56%                    | 8.8/4.2  | 13.7       |
| 6s   | NO N      | 0.952                | 11.5                 | 71.8                | 0.024                | 6.0     | 0.1  | <8.0           | 40%                    | 8.8/1.8  | 28.4       |
| 6t   | N          | 0.770                | 1.89                 | >100                | 0.017                | 3.5     | 0.6  | <8             | 45%                    | 8.8/NA   | 16.5       |
| 6u   | F3C        | 0.069                | 0.405                | 32.4                | 0.018                | 1.5     | 1.8  | <13            | 83%                    | 9.0/2.9  | 3.5        |

- **IC50** values obtained from BACE1 CFA.
- **IC50** values obtained from BACE2 CFA.
- **IC50** values obtained from CatD CFA.
- **IC50** values obtained from BACE1 WCA.
- Ratio from the MS-based quantiﬁcation of apical/basal and basal/apical transfer rates of a test compound at 2 μM across contiguous monolayers from MDRI-transfected MDCK cells.
- Hepatic clearance predicted from in vitro human microsomal stability study.
- CYP 2D6% inhibition determined in human microsomes using a probe CYP 2D6 substrate (dextromethorphan) and 3 μM of test compound.
- *pK_a* values measured.
- Measured IC50 in hERG-expressing CHO cells.

According to the table, the introduction of the diﬂuoromethyl group (6r) improves the BACE1 CFA potency and reduces the increase in MDR Er but simultaneously introduces a metabolic liability (HLM = 22 mL/min/kg). Replacement with a nitrile (6s) enhances BACE2 selectivity, but the MDR Er increases signiﬁcantly (MDR Er = 6.0). Use of an oxetane (6t) in place of the cyclopropane improves WCA potency and decreases log D but results in an elevated MDR Er ratio (MDR Er = 3.5) nearly equivalent to that of the parent cyclopropylamine 6p. In contrast, the CF3-cyclopropyl derivative 6u maintains good potency in both assay formats, exhibits a minimal eﬄux ratio and low clearance, and maintains excellent selectivity over CatD (470×) and modest selectivity over BACE2 (5.9×).

Although the decrease in pK_a imparted by the introduction of the cyclopropyl group in 6u (2.9 vs 3.8 for 6k) could contribute to the improved BACE1 CFA potency, it is not sufﬁcient to explain the 20-fold potency increase observed. To better rationalize this improvement, a co-crystal structure of compound 6u in BACE1 was obtained (PDB: ST1U). As shown in Figure 4, the thioamide and diﬂuorophenyl ring systems are oriented in a similar overall fashion to previously described BACE1 structures, with the diﬂuorophenyl occupying the S1 pocket as expected. As predicted by our initial design hypothesis, the benzylic amine substituent adopts an orientation orthogonal to the plane of the diﬂuorophenyl P1 group to optimally engage the carbonyl of Gly230. Interestingly, the triﬂuoromethyl group orients toward and ﬁlls the entrance to the S3 pocket, while the cyclopropyl substituent ﬁlls a small, lipophilic pocket at the back of the interface between the S1 and S3 pockets. Arguably, the significant...
improvement in BACE1 CFA potency observed for 6u is primarily achieved by optimally filling this cleft and locking the CF3 into the direction of the S3 pocket.

Overall, a liability of the series is P-gp-mediated efflux, likely driven in large part by the presence of a third hydrogen bond donor and exacerbated by increasing basicity at the benzylic amine (6o−6q, pK_a > 6, MDR Er 3.4−6.9). It was recognized that the installation of an electron-withdrawing group at the amine center aids in overcoming this issue, as illustrated by the difluoromethyl-substituted analogue 6r. Although the introduction of a nitrile substituent (6s) further lowers pK_a, the efflux liability increased, potentially because of the increase in polar surface area, a key factor for P-gp liability. In contrast, the relatively nonpolar trifluoromethyl group in 6u decreases pK_a sufficiently, resulting in a decreased efflux ratio.

Gratifyingly, 6u addresses a number of the key challenges that had emerged throughout the optimization of this series. As predicted by the in vitro transporter assay, 6u exhibits free access to the CNS compartment in mice (Cub/Cap = 1.0), as determined by time-course AUC. To assess in vivo potency, analogue 6u was dosed in wild-type mouse via subcutaneous administration at two doses, 10 and 100 mg/kg, to measure impact on levels of brain Aβ-42. At the lower dose, a small but significant decrease is observed at early time points, and at 100 mg/kg, robust lowering is observed out to 20 h postdose, providing a Cub/Cap of 31 nM for 25% Aβ lowering using previously described methodology (Figure 5).23

A goal from the outset had been to mitigate the hERG liability often observed for BACE1 inhibitors. Therefore, hERG IC_{50} values were generated for this second round of optimization. All analogues exhibit some affinity for hERG (IC_{50} 3.5−49 μM), although there is no correlation with increasing log D and decreasing pK_a. The hERG TI, as defined by comparing the hERG IC_{50} to the more robust and relevant measured C_{app}/C_{eff} value for BACE1, provides the most appropriate selectivity descriptor; for 6u, this corresponds to an in vivo hERG selectivity of 113X, satisfying our initial criteria.

Whereas 6u had achieved a balance of Aβ-lowering in brain, CNS penetration, and selectivity over hERG, it was, unfortunately, also characterized by significant inhibition of cytochrome P450 subtype 2D6 (CYP 2D6). CYP inhibition, in general, is undesirable due to the potential for drug−drug interactions (DDI) through inhibition of oxidative metabolism, but the significance is enhanced for subtype 2D6 because of significant polymorphism in 2D6 expression.24 Within this set of analogues, CYP 2D6 inhibition appears to be primarily driven by two main factors, namely, pK_a and the size of the benzylic amine substituent. Unfortunately, the CYP 2D6 values inversely correlate to pK_a such that analogues with decreased pK_a at the benzylic amine show greater inhibition, in direct opposition to the SAR utilized to obviate hERG and efflux transporter liabilities. In addition, increasing the size of the amine substituent appears to enhance CYP 2D6 inhibition, tracking with BACE1 potency. In an effort to more accurately gauge the CYP 2D6 inhibition, IC_{50} curves were generated for a subset of these compounds. In this assay, the unflanked trifluoroethylamine 6k showed only modest CYP 2D6 inhibition (IC_{50} = 14 μM), whereas 6u, much more potent at BACE1, significantly inhibited CYP 2D6 (IC_{50} = 157 nM). The intertwined SAR of CYP 2D6 inhibition, BACE1 potency, and transporter liability suggested that further tuning of the amine was unlikely to balance overall properties. Several alternative strategies were considered, including: (a) disrupting the favorable binding interactions between this series and CYP 2D6 while maintaining the high BACE1 affinity observed for 6u, and (b) building upon the modest BACE1 potency and absence of CYP 2D6 liability associated with 6k, but utilizing an alternative vector.

Disruption of the binding of this series to CYP 2D6 was informed by a recent report of a pyrazole-containing thioamidine series, which described a similar challenge with CYP 2D6 affinity (Figure 6).25

In this case, the fused pyrazole 7 is metabolized exclusively by CYP 2D6. Additionally, the primary metabolism product observed, the demethylated pyrazole, is a potent inhibitor of CYP 2D6 (IC_{50} = 0.37 μM). The CYP 2D6 affinity in this series was ultimately conquered through the installation of a substituent [fluoromethyl (8) or methyl (not shown)] adjacent to sulfur that disrupts binding to the CYP 2D6 active site.
The aminomethyl substituent in analogues positioning the di-thioamidines is anchored through an interaction with Glu-12 (Figure 7B) is oriented to the lipophilic pocket distal to the structures (Figures 7A and 7C) illustrates that each of the pairs of changes associated with this matched molecular pair, whereas the facility for reorientation in the monocyclic series is oriented toward the heme itself (Figure 7C). The orientations of the thioamidine rings in 6u and 12 are shifted, relative to that of the fused 7, to facilitate occupation of the distal pocket by the aminomethyl substituents. This reorientation creates greater distance between helix I and the difluorophenyl ring, and this shift is exacerbated slightly by the chiral methyl group in 12 (Figure 7B), which is easily accommodated. In contrast, the rigid architecture defined by the ring fusion of the thioamide and THP restricts the rotation of 7, as the compound occupies the cleft between Phe-120 and the residues Glu-244 and Phe-247 on helix G. The addition of a methyl or fluoromethyl group next to the sulfur of 7 then creates a direct clash with helix I, as evidenced by the lack of affinity of 8. Therefore, this restricted rotation underlies the pronounced substituent impact on CYP 2D6 affinity in the fused series, whereas the facility for reorientation in the monocyclic series 6u/12 minimizes the substituent effect.

Therefore, an alternative strategy to mitigate CYP 2D6 affinity in the aminomethyl series would exploit the restricted rotation imparted by ring fusion, precluding occupation of the distal, lipophilic pocket of CYP 2D6 by an aminomethyl substituent. In addition, from experience in previous series, the distal, lipophilic pocket of CYP 2D6 by an aminomethyl substituent impact on CYP 2D6 affinity in the aminomethyl series would exploit the restricted rotation underlies the pronounced substituent impact on CYP 2D6 affinity in the fused series, whereas the facility for reorientation in the monocyclic series 6u/12 minimizes the substituent effect.

Table 3. In Vitro Profile of 12

| compd | BACE1 CFA IC₅₀ (μM) | BACE2 CFA IC₅₀ (μM) | CatD CFA IC₅₀ (μM) | WCA IC₅₀ (μM) | MDR Er | log D | HLM f (mL/min/kg) | CYP 2D6 IC₅₀ (μM) | pK/1 | pK/2 | hERG g (μM) |
|-------|---------------------|---------------------|--------------------|---------------|--------|-------|----------------|-------------------|------|-------|-------------|
| 12    | 0.078               | 0.228               | >100               | 0.024         | 3.2    | 2.4   | 38             | 0.334             | 8.8/2.8 | 2.6   |

*IC₅₀ values obtained from CYP 2D6 IC₅₀ values obtained from BACE1 WCA. *Ratio from the MS-based quantification of apical/basal and basal/apical transfer rates of a test compound at 2 μM across contiguous monolayers from MDR1-transfected MDCK cells. Hepatic clearance predicted from in vitro human microsomal stability study. CYP 2D6 inhibition was obtained by measuring inhibition of 5 μM dextromorphan in pooled HLM (HL-MIX-102). Measured IC₅₀ in hERG-expressing CHO cells.

Figure 7. Comparison of the binding of 6u (A) and 12 (B) with the binding of 7 (C) in the active site of CYP 2D6.
Table 4. In Vitro Data for Compound 16

| compd | BACE1 CFA IC₅₀ (µM) | BACE2 CFA IC₅₀ (µM) | CatD CFA IC₅₀ (µM) | WCA IC₅₀ (nM) | MDR Er | log D | HLM CYP 2D6 IC₅₀ (µM) | hERG (µM) | C₅₀₂/C₅₀₁ (CYP) | C₅₀₂/C₅₀₁ (hERG) |
|-------|------------------|-----------------|-----------------|----------------|------|------|----------------|-----------|----------------|----------------|
| 16    | 0.077            | 0.295           | >100            | 0.006          | 2.3  | 1.1  | 29             | 9.1       | 7.91/3.80      | 4.3           |

“IC₅₀ values obtained from BACE1 CFA. IC₅₀ values obtained from BACE2 CFA. IC₅₀ values obtained from CatD CFA. IC₅₀ values obtained from BACE1 WCA. Ratio from the MS-based quantification of apical/basal and basal/apical transfer rates of a test compound at 2 µM across contiguous monolayers from MDR1-transfected MDCK cells. Hepatic clearance predicted from in vitro human microsomal stability study. CYP 2D6 inhibition was obtained by measuring inhibition of 5 µM dextromorphan in pooled HLM (HL-MIX-102). Measured IC₅₀ in hERG- expressing CHO cells.

As expected, the bicyclic fusion offered a 17× improvement of BACE1 CFA potency for 16 relative to the monocyclic 6k (Table 4), and 16 proved to be equipotent to 6u, reinforcing the observation that potency can be obtained in either of the two available vectors. Further, 16 maintained excellent CatD selectivity, similar selectivity over BACE1 (3.8×) and a balanced overall ADME profile, including, gratifyingly, a significantly diminished CYP 2D6 liability (IC₅₀ = 9.1 µM, 118-fold selectivity over BACE1 CFA) relative to the monocyclic analogues. A modest increase in efflux was observed relative to 6k, which translated into asymmetry in brain/plasma ratio (C₅₀₂/C₅₀₁ = 0.25, AUC ratio). Additionally, 16 exhibited excellent central Aβ-lowering in mice, with a measured brain C₅₀₂ for 25% lowering of Aβ/42 of 31 nM (Figure 8). The hERG value (IC₅₀ = 4.3 µM) was slightly improved relative to 6k, reflecting a better nominal selectivity (139×). However, accounting for brain asymmetry, the resultant hERG TI diminishes to 35× over the requisite plasma concentrations.

The observation of such disparate responses in the relative affinities to BACE1 and CYP 2D6 for 16 versus 6k was striking. The addition of the THP ring resulted in a 17× increase in potency at BACE1 while essentially having no impact on CYP 2D6 affinity (CYP 2D6 IC₅₀ for 6k = 14 µM). A crystal structure of 16 in BACE1 was generated and confirmed that, as expected, reorganization of the S2′ subpocket in BACE1 had occurred, driven by the rotation of Tyr71 to accommodate the THP ring and fluoromethyl substituent of 16 (Figure 9).

Therefore, selectivity is realized because this subpocket in BACE1 is responsive to increases in lipophilic bulk in this vector, whereas the same vector in CYP 2D6 directs toward the heme and has no impact on affinity. In addition, the ring fusion limits the requisite rotation of the thioamidine ring to optimally accommodate the aminomethyl substituent within the CYP 2D6 binding site. This discrepant response contrasts with the
parallel responsiveness at BACE1 and CYP 2D6 for small changes in the benzylic amine of the monocyclic series, wherein equivalent increases in lipophilicity afforded corresponding enhancements in affinity for both BACE1 and CYP 2D6 receptors (6k vs 6u, 20X BACE1, 9X CYP 2D6). Future BACE1 design efforts focused on avoiding CYP 2D6 liability will therefore target manipulation of the amidine “head group” architecture, rather than optimization of P3 substituents. Upon the basis of its attractive balance of potency, selectivity, and CNS penetration, 16 was selected for further safety profiling. The compound showed excellent in vitro selectivity, as gauged by screening against the broad CEREP bioprint panel (<25% inhibition at all targets at 10 μM). Subsequent advancement to long-term in vivo toxicology studies, including a 4-week arm at 100 mg/kg (30X AUC over C_{dd}), revealed no ocular findings, presumably reflecting the excellent in vitro selectivity observed for 16.

CONCLUSION

The optimization of a series of brain-penetrant BACE1 inhibitors with a novel aminomethyl linker has been described. This linker mitigates an aniline structural alert prevalent throughout a large contingent of literature BACE1 series. Careful modulation of the amine substituent aided in garnering a balanced profile, as the inherent efflux transporter liability and potency are dependent upon the resultant pK_{a}. A number of the more active compounds carried significant CYP 2D6 inhibition that correlated well with the size of the amine substituent. Unlike the fused THP series, substitution on the monocyclic thioamidine ring did not ameliorate CYP 2D6 inhibition, which was rationalized through examination of CYP 2D6 crystal structures. Filling the BACE1 S2’ subpocket by fusing a THP ring onto the monocyclic thioamidine ring improved BACE1 potency dramatically without impacting CYP 2D6 affinity. One such example (16) demonstrates good ADME balance, modest brain asymmetry, no DD1 potential, and good in vivo efficacy, illustrating that the aminomethyl linker can serve as a robust replacement to overcome the significant challenges and structural alerts inherent in the classical P1/P3 amide of previously described BACE1 inhibitors.

EXPERIMENTAL SECTION

Biology. In Vitro Pharmacology. sAPPβ Whole-Cell Assay (WCA). sAPPβ, the primary cleavage product of BACE1, was determined in H4 human neuroglioma cells overexpressing the wild-type human APP695. Cells were treated for 18 h with compound in a final concentration of 1% DMSO. sAPPβ levels were measured by ELISA with a capture APP N-terminal antibody (Affinity BioReagents, OMA1-03132), wild-type APPβ-specific reporter antibody p192 (Elan), and tertiary antirabbit-HRP (GE Healthcare). The colorimetric assay was read by an EnVision (PerkinElmer) plate reader.

BACE1 Enzyme Cell-Free Assay (FPA). Beta secretase-1 activity was measured with soluble BACE1 and the synthetic APP substrate Biotin-GLTNKTEESIEYVEFR-C[oregon green]KK-OH in the presence of compounds in a fluorescence polarization (FP) in vitro assay. Enzyme, substrate, and test compounds were incubated in 15 μL of 100 mM sodium acetate pH = 4.5 buffer containing 0.001% Tween-20 for 3 h at 37 °C. Following the addition of saturating immunopure streptavidin, fluorescence polarization was measured with a PerkinElmer EnVision plate reader (Ex685 nm/Em530 nm).

In Vivo Experiments. All procedures performed on animals in this study were in accordance with established guidelines and regulations, and were reviewed and approved by the Pfizer (or other) Institutional Animal Care and Use Committee. Pfizer animal care facilities that supported this work are fully accredited by AAALAC International.

Acute Treatment in Mice. Male 129/SVE wild-type mice (20–25 g) were in a nonfasted state prior to subcutaneous dosing with vehicle, or compound 6u or 16, using a dosing volume of 10 mL/kg in 5:5:90 DMSO:cremophor:saline vehicle. Mice (n = 5 per group) were sacrificed at 1, 3, 5, 7, 14, 20, and 30 h postdose. Whole blood samples (0.5–1.0 mL) were collected by cardiac puncture into ethylenediaminetetraacetic acid (EDTA)-containing tubes, and plasma was separated by centrifugation (1500g for 10 min at 3 °C). The generated plasma was distributed into separate tubes on wet ice for exposure measurements (50 μL) and Aβ42 analysis (remainder). CSF samples (8–12 μL) were obtained by cisterna magna puncture using a sterile 25 gauge needle and collected with a P-20 Eppendorf pipet.

Samples were analyzed using a Dissociation-Enhanced Lanthide Fluorescent Immunoassay (DELFI) platform Enzyme-Linked Immunosorbent Assay (ELISA). Configuration of the antibodies used in determining the level of Aβ40 and Aβ42 utilizes a common detect antibody (4G8) in combination with specific C-terminal antibodies for the 40 and 42 cleavage sites. For the Aβ40 assay, a concentration, and 15 μL was added to the Aβ40 assay plate in duplicate. CSF samples were diluted 1:8 in blocking buffer, and 15 μL was added to the Aβ40 assay plate in duplicate. Plates were incubated with phosphate-buffered saline containing 0.05% Tween-20 (PBS-T) and blocked with 75 μL of blocking buffer (1% BSA in PBS-T) for 2 h at 25 °C.

After washing the plates with PBS-T, rodent Aβ40/42 (California Peptide) or Aβ42 (California Peptide) standard was serially diluted in blocking buffer and 15 μL was applied to the plate in quadruplicate. Dried brain samples were reconstituted in 120 μL of blocking buffer, which corresponds to a 4.16–6.67-fold concentration. Then 15 μL of undiluted brain sample was added to the Aβ42 assay plate in triplicate or 15 μL of a 1:2 diluted brain sample was added to the Aβ40 assay plate in triplicate. Dried plasma samples were reconstituted in 40 μL of blocking buffer, which corresponds to a 3.5–4.38-fold concentration, and 15 μL was added to the Aβ40 assay plate in duplicate. CSF samples were diluted 1:8 in blocking buffer, and 15 μL was added to the Aβ40 assay plate in duplicate. Plates were incubated with sample or standards for 2 h at 25 °C. The plates were washed with PBS-T, and 15 μL of detecting antibody (4G8-Biotin, Covance), 200 ng/mL in blocking buffer, was added to each well, incubating for 2 h at 25 °C. The plates were then washed with PBS-T, and 15 μL of europium-labeled streptavidin (PerkinElmer), 50 ng/mL in blocking buffer, was added for a 1 h incubation in the dark at 25 °C. The plates were washed with PBS-T, and 15 μL of PerkinElmer Enhancement solution was added to each well with 20 μL incubation at rt. Plates were read on an EnVision plate reader using DELFI time-resolved fluorometry (Ex340/Em615), and samples were extrapolated against the standard curve using four-parameter logistics.

Measurement of human amyloid-β in plasma and CSF from P31/APP males
mice utilizes the same capture and detecting antibodies used for wild-type mice. Vehicle-treated samples from plasma and CSF were serially diluted to optimize sample dilution to the linear phase of an Aβ peptide standard curve. Aβ levels were measured using DELFIA ELISA.

Neuropharmacokinetic Studies in Male CD-1 Mice. The in-life and bioanalytical portions of these studies were conducted at BioDuro, Pharmaceutical Product Development Inc. (Beijing, China). Male CD-1 mice were obtained from PUMC, China. Mice received a 10 mg/kg subcutaneous (sc) dose of compounds 6u or 16. The doses were prepared in 5% DMSO/95% water containing (v/v) 0.5% methylcellulose (w/v) and delivered in a volume of 5 μL/kg. Animals were sacrificed in a CO₂ chamber. Blood, brain, and CSF samples were collected at 1, 4, and 7 h postdosing. Plasma was isolated after centrifugation. The plasma, brain, and CSF samples were stored at −80 °C prior to analysis.

Measurement of Fractions Unbound in Brain. The unbound fraction of each compound was determined in brain tissue homogenate using a 96-well equilibrium dialysis method as described by Kalvass et al.27 with the following exceptions. Brain homogenates were prepared from freshly harvested rat brains following dilution with a 4-fold volume of phosphate buffer and spiked with 1 μM compound. The homogenates were dialyzed against an equal volume (150 μL) of phosphate buffer at 37 °C for 6 h. Following the incubation, equal volumes (50 μL) of brain homogenate and buffer samples were collected and mixed with 50 μL of buffer or control homogenate, respectively, for preparation of mixed matrix samples. All samples were then precipitated with internal standard in acetonitrile (200 μL), vortexed, and centrifuged. Supernatants were analyzed using an LC-MS/MS assay. A dilution factor of 5 was applied to the calculation of brain fraction unbound.

Generic Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Assay for Exposure Measurements in Plasma, Brain, and CSF. Plasma, brain, and CSF were collected as described above and frozen at −80 °C until analysis by LC-MS/MS. Standard curves were prepared in respective matrix after serial dilution at a concentration of 1.0–2000 ng/mL (plasma and CSF) or 0.5–2000 ng/g (brain). For plasma, a 50 μL aliquot of sample was precipitated with 500 μL of MTBE containing an internal standard. Samples were vortexed for 1 min, then centrifuged at 3000 rpm for 10 min. The supernatant was transferred to a 96-well plate. Frozen brain tissue was weighed, and an 2-propanol:water (60:40) volume equivalent to 4 times the mass was added before homogenization in a bead beater (BioSpec Products Inc., Bartlesville, OK). A 50 μL aliquot of sample was precipitated with 500 μL of MTBE containing an internal standard. Samples were vortexed for 1 min, then centrifuged at 3000 rpm for 10 min. The supernatant was transferred to a 96-well plate. For CSF, a 50 μL aliquot of sample was precipitated with 500 μL of MTBE containing an internal standard. Samples were vortexed for 1 min, then centrifuged at 3000 rpm for 10 min. The supernatant (300 μL) was transferred to a 96-well plate. LC-MS/MS analysis was carried out using a high-performance liquid chromatography system consisting of tertiary Shimadzu LC20AD pumps (Shimadzu Scientific Technologies, Columbia, MD) with a CTC PAL autosampler (Leap Technologies, Columbia, MD) and a C18 column (Steffy, 150 × 2.1 mm, 3 μm). A mobile phase consisted of 80% mother liquor/20% glycerol, and subsequently crystals grown using the procedure described in ref21. The crystals were soaked at 0.6 mM for 3 h, transferred to a cryoprotectant comprised of 80% mother liquor/20% glycerol, and subsequently flash cooled in liquid nitrogen. X-ray diffraction data for compound 6u was collected at sector 17ID at the Advanced Photon Source (Argonne National Laboratory, Argonne, IL, USA) on a Pilatus 6 M detector at −170 °C. Data for compound 16 was collected in-house on a Rigaku FRE rotating anode X-ray generator equipped with a Rigaku Saturn 944 CCD detector. All data was processed using AUTOPROC28 and XD,$^{3}$ and subsequent data manipulation was performed using the CCP4 suite of programs.29 Initial structures were determined by rigid body refinement of a reference BACE structure, followed by restrained positional refinement in REFMAC.$^{3}$ Ligands were automatically fit to difference maps calculated after refinement in AUTOBUSTER,28 and all further refinement was performed in AUTOBUSTER. Data and refinement statistics are reported in Table S1 (see Supporting Information).

Crystallization of CYP 2D6. Structure characterization of the binding of 6u and 12 to CYP 2D6 was determined by X-ray crystallography (PDB: STFT and STFU, respectively). Crystals of a CYP 2D6 thiordinazine complex were prepared and soaked with an artificial mother liquor containing each of the compounds, with repeated transfers to fresh mother liquor to exchange the thiordinazine for the new compound as described previously.$^{3}$ Stock solutions (10×) were prepared in DMSO, and the final concentration of each compound in the artificial mother liquor was 5 mM. Data sets were
collected at the Stanford Synchrotron Light Source from single crystals at a temperature of 100 K. The data were integrated with XDS and merged, scaled, and processed using the CCP4 suite of programs. As the space group and unit cell were highly similar to that used to determine PDB 4XR4, molecular replacement by rigid body refinement was used for the four chains of the 4XR4 structure. Model building and refinement employed COOT and Phenix 1.9.18,19 respectively. The data processing and model refinement statistics are provided in Supporting Information, Table S2.

Chemistry. General Methods. Solvents and reagents were of reagent grade and were used as supplied by the manufacturer. All reactions were run under a N2 atmosphere. Organic extracts were routinely dried over anhydrous Na2SO4. Concentration refers to rotary evaporation under reduced pressure. Chromatography refers to flash chromatography using disposable RediSepR 4–120 g silica columns or Biotage disposable columns on a CombiFlash Companion or Biotage Horizon automatic purification system. Microwave reactions were carried out in a SmithCreator microwave reactor from Personal Chemistry. Purification by mass-triggered HPLC was carried out using Waters X-Terra Prep C18 columns, 5 μm, 30 mm × 100 mm. Compounds were pre-salted as TFA salts and diluted with 1 mL of water and dimethyl sulfoxide. Samples were purified by mass-triggered collection using a mobile phase of 0.1% trifluoroacetic acid in water and acetonitrile with a gradient of 100% aqueous to 100% acetonitrile over 60% gradient to afford a colorless oil in 60% yield. LCMS m/z 463.4 [M − H]+. 1H NMR (400 MHz, CDCl3) δ 7.88–7.95 (m, 1H), 7.36 (t, J = 8.8 Hz, 1H), 7.25 (dd, J = 2.2, 8.4 Hz, 1H), 6.83 (d, J = 9.4, 11.7 Hz, 1H), 6.37 (d, J = 8.2 Hz, 1H), 4.75–4.92 (m, 1H), 4.44–4.58 (m, 2H), 2.74–2.84 (m, 1H), 2.65–2.74 (m, 1H), 2.55 (dd, J = 12.3, 3.1 Hz, 1H), 2.18 (s, 3H), 1.97–2.10 (m, 1H), 1.69 (s, 3H), 1.53 (s, 9H).

tert-Butyl (S)-(4-(5-((Ethylinomethyl)ethyl)-2,4-difluoro phenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (5b). The title compound was prepared from 4 (150 mg, 0.45 mmol) and ethanolamine (27 mg, 0.61 mmol) according to a similar procedure as for the preparation of 5a in 88% yield. LCMS m/z 404.4 [M − H]+. 1H NMR (400 MHz, CDCl3) δ 7.18–7.27 (m, 1H), 6.81 (dd, J = 11.7, 9.4 Hz, 1H), 3.71–3.85 (m, 2H), 2.77–2.86 (m, 1H), 2.69–2.77 (m, 1H), 2.65 (q, J = 7.0 Hz, 2H), 2.51–2.60 (m, 1H), 1.98–2.09 (m, 2H), 1.70 (s, 3H), 1.50 (s, 9H).

tert-Butyl (S)-(4-(2,4-Difuuro-5-(isopropylaminomethyl) phenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (5c). The title compound was prepared from 4 (50 mg, 0.13 mmol) and propan-2-amine (12 mg, 0.20 mmol, 1.5 equiv) according to a similar procedure as for the preparation of 5a in 61% yield. LCMS m/z 414.4 [M − H]+. 1H NMR (400 MHz, CDCl3) δ 7.28–7.35 (m, 1H), 6.83 (dd, J = 11.7, 9.4 Hz, 1H), 3.77–3.90 (m, 2H), 2.78–2.89 (m, 2H), 2.66–2.75 (m, 1H), 2.60 (dt, J = 12.3, 3.1 Hz, 1H), 2.02–2.12 (m, 1H), 1.70 (s, 3H), 1.52 (s, 9H).

tert-Butyl (S)-(4-(2,4-Difluoro-5-((Dimethylaminomethyl) 2,4-difluorophenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (5d). The title compound was prepared from 4 (78 mg, 0.21 mmol) and dimethylamine (158 μL, 0.32 mmol, 2 M in methanol) according to a similar procedure as for the preparation of 5a in 59% yield. LCMS m/z 404.0 [M − H]+. 1H NMR (400 MHz, CDCl3) δ 7.25 (t, J = 8.8 Hz, 1H), 6.82 (dd, J = 11.9, 9.2 Hz, 1H), 3.39–3.54 (m, 2H), 2.79–2.89 (m, 2H), 2.67–2.77 (m, 1H), 2.59 (dt, J = 12.3, 3.1 Hz, 1H), 2.23 (s, 3H), 2.03–2.12 (m, 1H), 1.99–2.01 (m, 1H), 1.18 (s, 9H).

tert-Butyl (S)-(4-(2,4-Difuuro-5-(((R)-3-methoxy pyridinol-1-yl)methyl)phenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (5e). The title compound was prepared from 4 (160 mg, 0.43 mmol) and(R)-3-methoxypyridinol (66 mg, 0.65 mmol) according to a similar procedure as for the preparation of 5a in 59% yield. LCMS m/z 456.4 [M − H]+. 1H NMR (400 MHz, CDCl3) δ 7.27 (t, J = 8.59 Hz, 1H), 6.80 (dd, J = 8.98, 11.71 Hz, 1H), 3.86–3.96 (m, 2H), 3.57–3.73 (m, 1H), 2.77–2.78 (m, 1H), 2.59–2.70 (m, 1H), 2.50–2.59 (m, 2H), 2.46 (dd, J = 9.9, 3.7 Hz, 1H), 1.96–2.11 (m, 2H), 1.73–1.82 (m, 1H), 1.71 (s, 3H), 1.51 (s, 9H).

tert-Butyl (S)-(4-(2,4-Difuuro-5-(((2-methoxethyl)amino) methyl)phenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (5f). The title compound was prepared from 4 (115 mg, 0.31 mmol) and 2-methoxyethan-1-amine (35 mg, 0.47 mmol) according to a similar procedure as for the preparation of 5a in 72% yield. LCMS m/z 430.5 [M − H]+. 1H NMR (400 MHz, CDCl3) δ 7.21–7.33 (m, 1H), 6.82 (dd, J = 9.17, 11.90 Hz, 1H), 3.75–3.88 (m, 2H), 3.50 (t, J = 5.1 Hz, 2H), 3.30–3.39 (m, 3H), 2.69–2.88 (m, 4H), 2.59 (dt, J = 12.3, 3.1 Hz, 1H), 1.98–2.10 (m, 2H), 1.71 (s, 3H), 1.52 (s, 9H).

tert-Butyl (S)-(4-(2,4-Difuuro-5-(((3-methoxypropyl)amino) methyl)phenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (5g). The title compound was prepared from 4 (95 mg, 0.26 mmol) and 3-methoxypropyl-1-amine (34 mg, 0.38 mmol) according to a similar procedure as for the preparation of 5a in 58% yield. LCMS m/z 444.4 [M − H]+. 1H NMR (400 MHz, CDCl3) δ 7.25 (t, J = 8.7 Hz, 1H), 6.80 (dd, J = 11.7, 9.2 Hz, 1H), 3.74–3.84 (m, 2H), 3.43 (t, J = 6.0 Hz, 2H), 3.28–3.31 (m, 3H), 2.78–2.84 (m, 1H), 2.70 (t, J = 6.8 Hz, 2H), 2.53–2.62 (m, 1H), 1.99–2.08 (m, 1H), 1.76 (quin, J = 6.5 Hz, 2H), 1.69 (s, 3H), 1.49 (s, 9H).

tert-Butyl (S)-(4-(2,4-Difuuro-5-(((R)-1-methoxypropan-2 yl)amino)methyl)phenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (5h). The title compound was prepared from 4 (120 mg, 0.32 mmol) and the HCl salt of (R)-1-methoxypropan-2-amine (61 mg, 0.49 mmol) according to a similar procedure as for the preparation of 5a in 68% yield. LCMS m/z 444.4 [M − H]+.
NMR (400 MHz, CDCl₃) δ 7.22–7.31 (m, 1H), 6.80 (dd, J = 9.37, 11.71 Hz, 1H), 3.80 (s, 2H), 3.25–3.37 (m, 4H), 3.17–3.25 (m, 2H), 2.69–2.93 (m, 3H), 2.57 (dt, J = 12.2, 2.9 Hz, 1H), 1.96–2.10 (m, 1H), 1.69 (s, 3H), 1.50 (s, 9H), 1.03 (d, J = 6.2 Hz, 3H).

**ter-Butyl (S)-[4-[(2,3-di-O-benzyl-α-L-rhamopyranosyloxy)methyl]amino]-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl]carbamate (5f).** The title compound was prepared from 4 (100 mg, 0.27 mmol) and benzyl (1.1)-pentan-1-ylamino)methyl)-2,4-difluorophenyl)carbamate (12 mg, 0.02 mmol) according to a similar procedure as for the preparation of 5a in 31% yield. LCMS m/z 438.4 [M − H⁻]. ¹H NMR (400 MHz, CDCl₃) δ 7.23–7.34 (m, 1H), 6.82 (dd, J = 11.7, 9.4 Hz, 1H), 3.73–3.87 (m, 2H), 2.73–2.90 (m, 2H), 2.52–2.68 (m, 1H), 2.02–2.12 (m, 1H), 1.75–1.81 (m, 3H), 1.72 (s, 3H), 1.54 (s, 9H), 1.20–1.35 (m, 2H).

**ter-Butyl (S)-[4-(2,2,2-trifluoroethylamino)methyl]amino)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl]carbamate (5m).** The title compound was prepared from 4 (165 mg, 0.44 mmol) and the HCl salt of 2,2,2-trifluoro-N-methylthetan-1-amine (100 mg, 0.67 mmol) according to a similar procedure as for the preparation of 5a in 22% yield. LCMS m/z 468.3 [M − H⁻]. ¹H NMR (400 MHz, CDCl₃) δ 7.32 (t, J = 8.8 Hz, 1H), 6.84 (dd, J = 11.7, 9.0 Hz, 1H), 3.76 (s, 2H), 3.06 (q, J = 9.4 Hz, 2H), 2.80–2.88 (m, 1H), 2.69–2.80 (m, 1H), 2.58 (dt, J = 12.4, 3.3 Hz, 1H), 2.43 (s, 3H), 2.07 (J = 13.1, 5.3 Hz, 1H), 1.73 (s, 3H), 1.52 (s, 9H), 1.50 (s, 9H).

**ter-Butyl (S)-[4-(2,2-difluoroethylamino)-methyl]phenyl]-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl]carbamate (5k).** The title compound was prepared from 4 (235 mg, 0.61 mmol) and 2,2,2-trifluoroethan-1-amine (90 mg, 0.91 mmol) according to a similar procedure as for the preparation of 5a in 61% yield. LCMS m/z 454.4 [M − H⁻]. ¹H NMR (400 MHz, CDCl₃) δ 7.28 (t, J = 8.8 Hz, 1H), 6.83 (dd, J = 11.7, 9.4 Hz, 1H), 3.83–3.97 (m, 2H), 3.17 (q, J = 9.2 Hz, 2H), 2.79–2.89 (m, 1H), 2.66–2.79 (m, 1H), 2.57 (dt, J = 12.3, 3.1 Hz, 1H), 2.00–2.11 (m, 1H), 1.70 (s, 3H), 1.50 (s, 9H).
The aqueous solution was extracted with diethyl ether and the organic layer was dried over anhydrous sodium sulfate. The diethyl ether was evaporated to give a yellow oil, which was further purified by column chromatography on silica gel (2.5 × 40 cm) using hexane-ethyl acetate (3:1) as eluent to afford title compound 4a (63 mg, 0.14 mmol) as a white solid.

The title compound was prepared from 3a (142 mg, 0.355 mmol) according to a similar procedure as for the preparation of 4a in 63% yield. LCMS m/z 300.4 [M – H]⁻. 1H NMR (400 MHz, CDCl3) δ 7.41 (d, J = 1.6 Hz, 3H), 7.40 (t, J = 9.2 Hz, 1H), 7.24 (dd, J = 8.2, 2.3 Hz, 1H), 6.78 (dd, J = 11.7, 9.4 Hz, 1H), 6.33 (d, J = 8.6 Hz, 1H), 5.10 (t, J = 5.8 Hz, 1H), 4.40–4.56 (m, 2H), 2.89 (dd, J = 12.2, 6.2, 3.9 Hz, 1H), 2.59 (dt, J = 11.5, 3.5 Hz, 1H), 2.31–2.42 (m, 1H), 2.18 (s, 3H), 1.83 (dd, J = 14.0, 10.7, 3.7 Hz, 1H), 1.57 (d, J = 1.2 Hz, 3H).

The title compound was prepared from 5b (97 mg, 0.22 mmol) according to a similar procedure as for the preparation of 4a in 56% yield. LCMS m/z 334.4 [M – H]⁻. 1H NMR (400 MHz, CDCl3) δ 7.33 (t, J = 9.0 Hz, 1H), 6.76 (dd, J = 11.7, 9.4 Hz, 1H), 3.78–3.91 (m, 2H), 3.18 (d, J = 12.0, 6.3, 3.9 Hz, 1H), 2.81–2.91 (m, 1H), 1.87 (d, J = 14.0, 10.4, 3.7 Hz, 1H), 1.57 (d, J = 1.2 Hz, 3H).

The title compound was prepared from 5c (34 mg, 0.08 mmol, 1 equiv) in 70% yield. LCMS m/z 290.4 [M – H]⁻. 1H NMR (400 MHz, CDCl3) δ 7.84 (d, J = 8.5 Hz, 1H), 6.92 (d, J = 9.0, 11.5 Hz, 1H), 4.78 (s, 2H), 4.15–4.32 (m, 2H), 3.29 (sept, J = 6.5 Hz, 1H), 2.96–3.10 (m, 1H), 2.78–2.81 (m, 2H), 2.10–2.24 (m, 1H), 1.82 (s, 3H), 1.49 (dd, J = 7.5, 6.8 Hz, 6H).

The title compound was prepared from 5d (59 mg, 0.15 mmol) according to a similar procedure as for the preparation of 4a in 60% yield. LCMS m/z 314.4 [M – H]⁻. 1H NMR (400 MHz, CDCl3) δ 8.07 (t, J = 8.5 Hz, 1H), 6.92 (d, J = 9.0, 11.5 Hz, 1H), 4.78 (s, 2H), 4.15–4.32 (m, 2H), 3.29 (sept, J = 6.5 Hz, 1H), 2.96–3.10 (m, 1H), 2.78–2.81 (m, 2H), 2.10–2.24 (m, 1H), 1.82 (s, 3H), 1.49 (dd, J = 7.5, 6.8 Hz, 6H).
(5)-(4,2,4-Difluoro-5-((1,1,3-trifluoro-2-methylpropan-2-yl)amino)methylphenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (6a). The title compound was prepared from 5a (39 mg, 0.08 mmol) according to a similar procedure as for the preparation of 6a in 38% yield. LCMS m/z 382.4 [M – H]–. 1H NMR (400 MHz, CDCl3) δ 7.41 (t, J = 9.0 Hz, 1H), 6.71–6.81 (m, 1H), 3.78–3.92 (m, 2H), 2.98 (ddd, J = 12.1, 6.8, 3.7 Hz, 1H). 2.69 (ddd, J = 12.3, 10.3, 3.9 Hz, 1H), 2.35 (ddd, J = 14.0, 6.8, 3.7 Hz, 1H). 2.19 (ddd, J = 14.0, 10.3, 3.7 Hz, 1H) 1.52–1.62 (m, 3H), 0.78–0.86 (m, 4H).

(5)-(1-(5-Amino-4-methyl-5,6-dihydro-4H-1,3-thiazin-4-yl)-2,4-difluorobenzyl) amino)cyclopropane-1-carbonitrile (6a). The title compound was prepared from 5a (51 mg, 0.12 mmol) according to a similar procedure as for the preparation of 6a in 48% yield. LCMS m/z 337.4 [M – H]–. 1H NMR (400 MHz, CDCl3) δ 7.35 (t, J = 9.0 Hz, 1H), 6.69–6.80 (m, 1H), 5.45–5.79 (m, 1H), 4.29 (br s, 2H), 3.96 (d, J = 7.0 Hz, 2H), 2.95 (ddd, J = 12.1, 6.6, 3.9 Hz, 1H), 2.67 (dd, J = 12.1, 10.5, 3.5 Hz, 1H). 2.35 (dd, J = 14.0, 6.6, 3.5 Hz, 1H), 2.05 (dd, J = 6.8 Hz, 1H), 1.88 (dd, J = 14.0, 10.4, 3.7 Hz, 1H), 1.54–1.61 (m, 3H), 1.16–1.25 (m, 2H), 1.00–1.09 (m, 2H).

(5)-(4,2,4-Difluoro-5-((3,3,3-trifluoropropyl)amino)methylphenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (6a).

The title compound was prepared from 5a (75 mg, 0.16 mmol) according to a similar procedure as for the preparation of 6a in 19% yield. LCMS m/z 368.3 [M – H]–. 1H NMR (400 MHz, CDCl3) δ 7.32–7.43 (m, 1H), 6.74–6.86 (m, 1H), 3.82–4.01 (m, 2H), 3.27–3.27 (m, 1H), 2.89–3.05 (m, 2H), 2.61–2.75 (m, 2H), 2.30–2.42 (m, 1H). 1.81–1.96 (m, 1H), 1.59–1.63 (m, 3H), 1.25 (d, J = 6.6 Hz, 3H).

(5)-(2,4-Difluoro-5-(((3,3,3-trifluoropropyl)amino)methyl)phenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (6d). The title compound was prepared from 5a (58 mg, 0.14 mmol) according to a similar procedure as for the preparation of 6a in 19% yield. LCMS m/z 312.3 [M – H]–. 1H NMR (400 MHz, CDCl3) δ 7.32 (t, J = 9.0 Hz, 1H), 6.77 (dd, J = 11.9, 9.6 Hz, 1H), 3.79–3.89 (m, 2H), 2.96 (ddd, J = 12.1, 6.4, 3.7 Hz, 1H), 2.67 (dd, J = 12.2, 10.4, 3.7 Hz, 1H), 2.25–2.39 (m, 3H), 1.89 (ddd, J = 14.0, 10.4, 3.7 Hz, 1H), 1.59 (d, J = 1.6 Hz, 3H).

(5)-(4-(3-Cyclopropylmethyl)amino)methyl)-4,2,4-difluorophenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (6a).

The title compound was prepared from 5a (38 mg, 0.09 mmol) according to a similar procedure as for the preparation of 6a in 34% yield. LCMS m/z 327.4 [M – H]–. 1H NMR (400 MHz, CDCl3) δ 7.32 (t, J = 9.2 Hz, 1H), 6.75 (ddd, J = 11.9, 9.6, 11.9 Hz), 3.80 (s, 2H), 2.96 (ddd, J = 12.2, 6.7, 3.7 Hz, 1H), 2.67 (dd, J = 12.3, 10.3, 3.9 Hz, 1H), 2.39–2.52 (m, 2H), 2.34 (ddd, J = 14.0, 6.7, 3.9 Hz, 1H), 1.89 (dd, J = 14.0, 10.4, 3.7 Hz, 1H), 1.58 (d, J = 1.6 Hz, 3H), 0.88–1.03 (m, 1H), 0.41–0.50 (m, 2H), 0.04 to 0.13 (m, 2H).

(5)-(4-(1-(1-Difluoromethyl)cyclopropyl)amino)methyl)-2,4-difluorophenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (6a).

The title compound was prepared from 5a (38 mg, 0.08 mmol) according to a similar procedure as for the preparation of 6a in 38% yield. LCMS m/z 362.4 [M – H]–. 1H NMR (400 MHz, CDCl3) δ 7.35 (t, J = 9.0 Hz, 1H), 6.69–6.80 (m, 1H), 5.45–5.79 (m, 1H), 4.29 (br s, 2H), 3.96 (d, J = 7.0 Hz, 2H), 2.95 (ddd, J = 12.1, 6.6, 3.9 Hz, 1H), 2.67 (dd, J = 12.1, 10.5, 3.5 Hz, 1H). 2.35 (dd, J = 14.0, 6.6, 3.5 Hz, 1H), 2.05 (dd, J = 6.8 Hz, 1H), 1.88 (dd, J = 14.0, 10.4, 3.7 Hz, 1H), 1.54–1.61 (m, 3H), 0.78–0.86 (m, 4H).

(5)-(1-(5-Amino-4-methyl-5,6-dihydro-4H-1,3-thiazin-4-yl)-2,4-difluorobenzyl) amino)cyclopropane-1-carbonitrile (6a).

The title compound was prepared from 5a (39 mg, 0.12 mmol) according to a similar procedure as for the preparation of 6a in 48% yield. LCMS m/z 337.4 [M – H]–. 1H NMR (400 MHz, CDCl3) δ 7.34 (t, J = 9.0 Hz, 1H), 6.78 (dd, J = 11.7, 9.4 Hz, 1H), 3.90–4.02 (m, 2H), 2.95 (ddd, J = 12.2, 6.5, 3.9 Hz, 1H). 2.69 (dd, J = 12.1, 10.5, 3.5 Hz, 1H), 2.36 (dd, J = 14.0, 6.6, 3.9 Hz, 1H). 1.88 (dd, J = 14.0, 10.5, 3.5 Hz, 1H), 1.54–1.61 (m, 3H), 1.16–1.25 (m, 2H), 1.00–1.09 (m, 2H).

(5)-(4,2,4-Difluoro-5-(((3,3,3-trifluoropropyl)oxytetralyn)-3-yl)methyl)phenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (6a).
under reduced pressure. Flash column chromatography of the organic crude, using a gradient of EtOAc in heptanes (0–100%) afforded 13 (14.5 g, 32.5 mmol, 85% yield) as a white solid. 

\[
N-(4aR,6R,8aS)-8a-(5-(Aminomethyl)-2,4-difluorophenyl)-6-(fluoromethyl)-4a,5,6,8a-hexahydropyrano[3,4-d][1,3]thiazin-2-yl)benzamide (15). In a pressure vessel equipped with a stir bar, under N₂, an acetonitrile (225 mL, anhydrous) solution of crude was partitioned between H₂O (150 mL) and EtOAc (150 mL). The resulting solution was stirred at 70 °C until complete consumption of starting material was observed. The reaction mixture was filtered through a Celite pad, which was then washed with MeOH. The filtrates were concentrated under reduced pressure and redissolved in MeOH (300 mL). The resulting solution was treated with HCl (4 M in dioxane, 162.6 mL, 650 mmol). Once the Boc-removal was complete, the reaction vessel was placed under a H₂ atmosphere of 70 PSI and was left stirring at rt until complete consumption of starting material was observed. The reaction mixture was then filtered through a Celite pad, which was then washed with 3x with MeOH. The filtrates were concentrated under reduced pressure and redissolved in MeOH (300 mL). The resulting solution was treated with HCl (4 M in dioxane, 162.6 mL, 650 mmol). Once the Boc-deprotection was complete, the reaction mixture was concentrated under reduced pressure, redissolved in CH₂Cl₂, and treated with Na₂SO₄, stirred at rt for 15 min. After that time, the layers were separated and the aq layer was washed with CH₂Cl₂ (3 x 200 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Flash column chromatography of the organic crude, using a gradient of MeOH in CH₂Cl₂ (0–5%), afforded 14 (9.0 g, 20 mmol, 61% yield) as a white solid.

\[
(4aR,6R,8aS)-8a-(2,4-Difluoro-5-(((2,2,2-trifluoroethylamino)methyl)phenyl)-6-(fluoromethyl)-4a,5,6,8a-hexahydropyrano[3,4-d][1,3]thiazin-2-amine (16). In a pressure vessel equipped with a stir bar, under N₂, an acetonitrile (225 mL, anhydrous) solution of 15 (4.76 g, 10.6 mmol) was treated with TEA (2.21 mL, 15.9 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (2.29 mL, 15.9 mmol). The resulting solution was stirred at 70 °C until complete consumption of starting material was observed. The reaction mixture was cooled down to rt and concentrated under reduced pressure. The organic crude was partitioned between H₂O (150 mL) and EtOAc (150 mL). The layers were separated, and the aq layer was back-extracted with EtOAc (2x). The combined organics were then washed with brine (1x), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to afford N-(4aR,6R,8aS)-8a-(2,4-difluoro-5-(((2,2,2-trifluoroethylamino)methyl)phenyl)-6-(fluoromethyl)-4a,5,6,8a-hexahydropyrano[3,4-d][1,3]thiazin-2-yl)benzamide (4.58 g, 8.62 mmol, 84% yield) as a yellow solid.

**DBU (0.947 mL, 6.01 mmol) was added to a methanol solution (407 mL, anhydrous) of N-(4aR,6R,8aS)-8a-(2,4-difluoro-5-(((2,2,2-trifluoroethylamino)methyl)phenyl)-6-(fluoromethyl)-4a,5,6,8a-hexahydropyrano[3,4-d][1,3]thiazin-2-yl)benzamide (4.58 g, 8.62 mmol). The resulting solution was heated to 70 °C until complete consumption of starting material was observed. The reaction mixture was concentrated under reduced pressure. Flash column chromatography of the organic crude, using a gradient of MeOH in CH₂Cl₂ (0–5%), afforded 16 (2.60 g, 6.08 mmol, 71% yield) as a white foam. **H NMR (400 MHz, CDCl₃) δ 1.43 – 1.51 (m, 1H, 1H) 1.87 (qd, J = 12.3, 2.5 Hz, 1H) 2.62 (dd, J = 12.1, 2.3 Hz, 1H) 2.87 – 3.00 (m, 2H) 3.08 – 3.21 (m, 2H) 3.82 (d, J = 11.3 Hz, 1H) 3.86 – 3.99 (m, 3H) 4.10 (dd, J = 11.1, 2.2 Hz, 1H) 4.34 – 4.46 (m, 1H) 4.46 – 4.58 (m, 1H) 4.94 (br s, 1H) 6.81 (dd, J = 11.7, 9.4 Hz, 1H) 7.34 (t, J = 8.8 Hz, 1H). **C NMR (400 MHz, CDCl₃) δ 26.55, 28.46, 28.97, 46.29, 58.31, 75.37, 84.36, 86.06, 104.92, 122.18, 124.95, 132.54, 157.17, 159.08, 159.69, 161.56.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b01451.

**Accession Codes**

Atomic coordinates and structure factors for the following BACE1 crystal structures have been deposited with the RCSB: compound 6u (PDB: STFU), compound 16 (PDB: STIF). Atomic coordinates and structure factors have been deposited with the RCSB for crystal structures of CYP 2D6 complexed with compound 6u (PDB: STFT), and with compound 12 (PDB: STFU).

**AUTHOR INFORMATION**

* Corresponding Author

**ORCID**

Christopher R. Butler: 0000-0002-9387-5011

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We thank David Karanian for toxicology expertise, Leslie Pustilnik, Stephen Noell, Carol Menard, and Theresa Dickinson for the generation of in vitro data and assay reagents, as well as Romelia Salomon-Ferrer, Katherine Brighty, and Pat Verhoest for helpful discussion in manuscript preparation. Use of the IMCA-CAT beamline 17-ID at the Advanced Photon Source was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Hauptman-Woodward Medical Research Institute. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract no. DE-AC02-06CH11357. This work was supported in part by NIH grant GM031001. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Data collection for crystals of the P450 2D6 complexes was carried out at the Stanford Synchrotron Radiation Lightsource, a national user facility operated by Stanford University on behalf of the United States Department of Energy, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. The Stanford Synchrotron Radiation Lightsource Structural Molecular Biology Program is supported by the United States Department of Energy, Office of Biological and Environmental Research and by the National Center for Research Resources, Biomedical Technology Program, and NIGMS (including P41GM103933) of the National Institutes of Health.

**ABBREVIATIONS USED**

BACE1, β-secretase; sAPPβ, N-terminal ectodomain of APP; C99, C-terminal fragment; hERG, human ether-a-go-go-related gene; CatD, cathepsin D; RPE, retinal pigment epithelium; P-gp, P-glycoprotein; C\textsubscript{250}/C\textsubscript{0} F, ratio of unbound concentration in brain to the unbound concentration in plasma; CFA, cell-free assay; WCA, whole-cell assay; HLM, human liver microsomes; LipE, lipophilic efficiency; EWG, electron-withdrawing group; Er, efflux ratio; C\textsubscript{eff} efficacious concentration; DELFIA, dissociation-enhanced lanthanide fluorescent immunoassay
(1) Hardy, J.; Allsop, D. Amyloid deposition as the central event in the aetiology of Alzheimer’s disease. Trends Pharmacol. Sci. 1991, 12, 383–388.

(2) Tanzi, R. E.; Bertram, L. Twenty years of the Alzheimer’s disease amyloid hypothesis: A genetic perspective. Cell 2005, 120, 545–555.

(3) De Strooper, B. Proteases and proteolysis in Alzheimer Disease: a multifactorial view on the disease process. Physiol. Rev. 2010, 90, 465–494.

(4) Vassar, R.; Kovacs, D. M.; Yan, R.; Wong, P. C. The beta-secretase enzyme BACE in health and Alzheimer’s disease: regulation, cell biology, function, and therapeutic potential. J. Neurosci. 2009, 29, 12787–12794.

(5) Marks, N.; Berg, M. J. BACE and gamma-secretase characterization and their sorting as therapeutic targets to reduce amyloidogenesis. Neurochem. Res. 2010, 35, 181–210.

(6) Jonsson, T.; Atwal, J. K.; Steinberg, S.; Snaedal, J.; Jonsson, P. V.; Bjornsson, S.; Stefansson, H.; Sulem, P.; Guddbjartsson, D.; Maloney, J.; Hoyte, K.; Gustafson, A.; Liu, Y.; Lu, Y.; Bhangale, T.; Graham, R. R.; Huttenlocher, J.; Bjornsodtrit, G.; Andresen, O. A.; Jonsson, E. G.; Palotie, A.; Behrens, T. W.; Magnusson, O. T.; Kong, A.; Thorsteinsdottir, U.; Watts, R. J.; Stefansson, K. A mutation in APP protects against Alzheimer’s disease and age-related cognitive decline. Nature 2012, 488, 96–99.

(7) Evin, G.; Hince, C. BACE1 as a therapeutic target in Alzheimer’s disease: rationale and current drugs. Drugs Aging 2013, 30, 755–764.

(8) (a) Sanguinetti, M. C.; Tritiun-Fioruzi, M. hERG potassium channels and cardiac arrhythmia. Nature 2006, 440, 463–469.

(b) Sanguinetti, M. C.; Jiang, C.; Curran, M. E.; Keating, M. T. A mechanistic link between an inherited and an acquired cardiac arrhythmia: hERG encodes the IKr potassium channel. Cell 1995, 81, 299–307. (c) Roden, D. M. Drug-induced prolongation of the QT interval. N. Engl. J. Med. 2004, 350, 1013–1022.

(9) May, P. C.; Dean, R. A.; Lowe, S. L.; Martenyi, F.; Sheehan, S. M.; Baggs, L. N.; Monk, S. A.; Mathes, B. M.; Morgert, D. J.; Watson, B. M.; Stout, S. L.; Timm, D. E.; Smith LaBell, E.; Gonzales, C. R.; Nakano, M.; Jhee, S. S.; Yen, M.; Ereshefsky, L.; Lindstrom, T. D.; Calligaro, D. O.; Cocke, P. J.; Hall, D. G.; Friedrich, S.; Citron, M.; Audia, J. E. Robust central reduction of amyloid-β in humans with an orally available, non-peptidic beta-secretase inhibitor. J. Neurosci. 2011, 31, 16507–16516.

(10) (a) Waring, M. J.; Johnstone, C. A quantitative assessment of hERG liability as a function of lipophilicity. Bioorg. Med. Chem. Lett. 2007, 17, 1759–1764. (b) Jamieson, C.; Moir, E. M.; Rankowiecki, Z.; Wishart, G. Medicinal chemistry of hERG optimiztions: highlights and hang-ups. J. Med. Chem. 2006, 49, 5029–5046.

(11) Rochin, L.; Hurbain, I.; Serneels, L.; Fort, C.; Watt, B.; Leblanc, P.; Martino, A.; Alonso de Diego, S. A.; Oehlrich, D.; Prokopcova, H.; Alonso, J.; Lundkvist, J.; Noeidjil, E.; Eketjail, S.; Ramberg, B.; Bueters, T.; Agerman, K.; Juréus, A.; Svensson, S.; Berg, S.; Fålling, J.; Landahl, U. Revisiting the peripheral sink hypothesis: inhibiting BACE1 activity in the periphery does not alter β-amyloid levels in the CNS. J. Neurochem. 2015, 132, 477–486.

(12) Shalaeva, M.; Kenseth, J.; Lombardo, F.; Bastin, A. Measurement of dissociation constants (pK values) of organic compounds with multilayered capillary electrophoresis using aqueous and cosolvent buffers. J. Pharm. Sci. 2008, 97, 2581–2606.

(13) Shalaeva, M.; Kenseth, J.; Lombardo, F.; Bastin, A. Measurement of dissociation constants (pK values) of organic compounds with multilayered capillary electrophoresis using aqueous and cosolvent buffers. J. Pharm. Sci. 2008, 97, 2581–2606. (23) Lu, Y.; Riddell, D.; Hajos-Koroczk, E.; Bales, K.; Wood, M.; Nolan, C. E.; Robshaw, A.; Eshel, Z.; Zhang, L.; Leung, L.; Becker, S. L.; Tseng, E.; Barricklow, J.; Miller, E. H.; Osgood, S.; O’Neill, B. T.; Brodny, M. A.; Johnson, D. S.; Pettersson, M. Cerebrospinal fluid amyloid-β (Aβ) as an effector biomarker for brain Aβ lowering verified by quantitative prinicpal analyses. J. Pharmacol. Exp. Ther. 2012, 342, 366–375.
(24) (a) Rendic, S.; Carlo, F. J. D. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug Metab. Rev. 1997, 29, 413−580. (b) Teh, L. K.; Bertilsson, L. Pharmacogenomics of CYP 2D6: molecular genetics, interethnic differences and clinical importance. Drug Metab. Pharmacokinet. 2012, 27, 55−67. (c) Bertilsson, L.; Dahl, M.-L.; Dalen, P.; Al-Shurbaji, A. Molecular genetics of CYP 2D6: Clinical relevance with focus on psychotropic drugs. Br J. Clin. Pharmacol. 2002, 53, 111−122.

(25) Brodney, M. A.; Beck, E. M.; Butler, C. R.; Barreiro, G.; Johnson, E. F.; Riddell, D.; Parris, K.; Nolan, C. E.; Fan, Y.; Atchison, K.; Gonzales, C.; Robshaw, A. E.; Doran, S. D.; Bundesmann, M. W.; Buzon, L.; Dutra, J.; Henegar, K.; LaChapelle, E.; Hou, X.; Rogers, B. N.; Pandit, J.; Lira, R.; Martinez-Alsina, L.; Mikochik, P.; Murray, J. C.; O’Neill, B. T. Utilizing structures of CYP 2D6 and BACE1 complexes to reduce risk of drug−drug interactions with a novel series of centrally efficacious BACE1 inhibitors. J. Med. Chem. 2015, 58, 3223−3252.

(26) Compound 13 was prepared in analogous fashion to ref 21. Preparation and characterization information is included in the Supporting Information.

(27) Kalvass, J. C.; Maurer, T. S. Influence of nonspecific brain and plasma binding on CNS exposure: implications for rational drug discovery. Biopharm. Drug Dispos. 2002, 23, 327−338.

(28) Kutchorovskiy, J.; Friis, S.; Asmild, M.; Taboryski, R.; Pedersen, S.; Vestegaard, R. K.; Jacobsen, R. B.; Krzywkoowski, K.; Schroder, R. L.; Ljungstrom, T.; Helix, N.; Sorensen, C. B.; Bech, M.; Willumsen, N. J. Characterization of potassium channel modulators with QPatch automated patch-clamp technology: system characteristics and performance. Assay Drug Dev. Technol. 2003, 1, 685−693.

(29) Bridges, K. G.; Chopra, R.; Lin, L.; Svenson, K.; Am, A.; Jin, G.; Cowling, R.; Lovering, F.; Akopian, T. N.; DiBlasio-Smith, E.; Annis-Freeman, B.; Marvell, T. H.; LaVallie, E. R.; Zollner, R. S.; Bard, J.; Somers, W. S.; Stahl, M. L.; Kriz, R. W. A novel approach to identifying β-secretase inhibitors: Biso-statine peptide mimetics discovered using structure and spot synthesis. Peptides (N. Y., NY, U. S.) 2006, 27, 1877−1885.

(30) Vonrhein, C.; Flensburg, C.; Keller, P.; Sharff, A.; Smart, O.; Paciorek, W.; Womack, T.; Bricogne, G. Data processing and analysis with the autoPROC toolbox. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2011, 67, 293−302.

(31) Kabsch, W. XDS. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2010, 66, 125−132.

(32) (a) French, S.; Wilson, K. On the treatment of negative intensity observations. Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr. 1978, A34, S17−S25. (b) Collaborative computational project, number 4. The CCP4 suite: programs for protein crystallography. Acta Crystallogr., Sect. D: Biol. Crystallogr. 1994, 50, 760−763.

(33) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr., Sect. D: Biol. Crystallogr. 1997, D53, 240−255.

(34) Brando, G.; Blanc, E.; Brandl, M.; Brando, C.; Keller, P.; Paciorek, W.; Roversi, P.; Smart, O. S.; Vonrhein, C.; Womack, T. O. BUSTER; Global Phasing Ltd: Cambridge, UK, 2011.

(35) Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2004, D60, 2126−2132.

(36) Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2010, 66, 213−221.