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Discovery of S3-truncated, C-6 heteroaryl substituted aminothiazine β-site APP cleaving enzyme-1 (BACE1) inhibitors

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Supporting Information Placeholder

ABSTRACT: Truncation of the S3 substituent of the biaryl aminothiazine **2**, a potent BACE1 inhibitor, led to a low molecular weight aminothiazine **5** with moderate activity. Despite its moderate activity, compound **5** demonstrated significant brain Aβ reduction in rodents. The metabolic instability of **5** was overcome by the replacement of the 6-dimethylisoxazole, a metabolic soft spot, with a pyrimidine ring. Thus, truncation of the S3 substituent represents a viable approach to the discovery of orally bioavailable, brain-penetrant BACE1 inhibitors.

INTRODUCTION

There is much evidence to suggest that β-amyloid (Aβ) peptide, particularly the longer 42 amino acid form Aβ42, plays a critical role in the progression of Alzheimer's disease (AD).[1-3] For example, human genetic mutations such as the Swedish mutation result in increased β-secretase processing of β-

amyloid precursor protein (APP), elevated Aβ levels, and ultimately aggressive early onset AD.[4] A more recent study reported a variant of the APP gene (APPA673T) that confers protection against AD in certain humans. A673T substitution in APP reduces production of Aβ peptides secreted from heterologously transfected cells, supporting the hypothesis that decreases in Aβ can protect against AD, and conversely that increases in Aβ may underlie the pathology of AD.[5] Aβ is derived from APP by proteolysis. Cleavage of APP by β-site APP cleaving enzyme-1 (BACE1) results in shedding of the APP ectodomain, and the remaining membrane bound C-terminal fragment, C99, is further processed by γ-secretase to produce Aβ. Thus, inhibition of BACE1 to reduce Aβ production is a promising approach to test the amyloid hypothesis.[6-10]

In 2009, Eli Lilly reported that **1** (LY2811376, Figure 1), a biaryl aminothiazine BACE1 inhibitor, demonstrated sustained brain Aβ reduction in healthy volunteers upon oral administration.[11,12] However, this compound exhibited only modest enzyme inhibitory activity (IC_{50} = 0.27 μM). Recently, we disclosed a related series of compounds with potency improved by 34-fold via introduction of a C-6 dimethylisoxazole substituent onto the thiazine core (compound **2**, IC_{50} = 8 nM).[13] This more potent series benefits from the two hydrogen-bond interactions between the isoxazole and threonine 72 and glutamine 73 of the flap backbone as revealed by the co-crystal structure of **2** bound to the BACE1 active site. Despite its potency, compound **2** produced only modest brain Aβ reduction in rodents.[13] The lack of robust *in vivo* activity was ascribed to poor brain penetration and metabolic instability of **2**.[13] Both hurdles were overcome by the replacement of the metabolically labile pyrimidine ring in **2** with a methoxypyrazine-2-carboxamide side chain to afford carboxamide **3** (Figure **1**), which exhibited potent BACE inhibitory activity (IC_{50} = 0.7 nM), and elicited robust brain Aβ reduction in rodents. Herein, we describe another approach to address the two major issues confronting thiazine **2**.

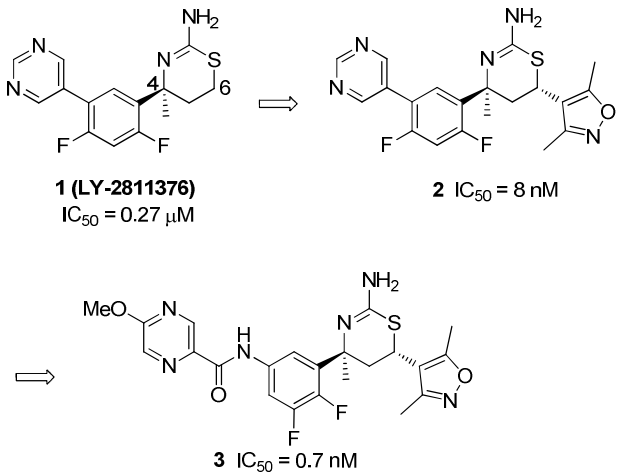


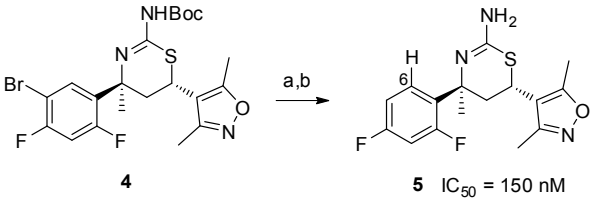
Figure 1. Previous work

RESULTS AND DISCUSSION

With regard to the modest brain penetration of **2**, a common approach to improve brain penetration is to reduce molecular weight and polar surface area. Thus, we probed truncation of the biaryl aminothiazine **2** by removing the pyrimidine ring in the S3 pocket. This approach was also expected to improve the metabolic stability, as *in vitro* studies had identified oxidation of the pyrimidine ring in **2** as a metabolic soft spot. We prepared **5** from known bromide **4**[13] in a straightforward fashion (Scheme 1). The truncated aminothiazine **5** exhibited modest BACE1 inhibitory activity (IC_{50} = 150 nM). The 20-fold reduction in potency, which was remarkably consistent with that observed in the 6-unsubstituted aminothiazine series,[12] can be rationalized by examining the co-crystal structures of both **2**[13] and **5**[14] bound to the BACE1 active site (Figure 2). The bicyclic aminothiazine core engaged the catalytic dyad of BACE1 while the fluorophenyl ring bound in the S1 pocket; the dimethylisoxazole formed two hydrogen-bonds with the flap backbone,[15] and a potential C-H hydrogen-bond also exists between the C-6 hydrogen of the phenyl (see **5**, Scheme 1) and the carbonyl of Gly230 on the backbone. The pyrimidine ring of biaryl aminothiazine **2** is projected into the opening of the S3 pocket where it makes both hydro-

phobic contacts and interacts with the carbonyl of Ser229 via a bridging water molecule; consequently, its removal reduces activity.

Scheme 1. Synthesis of compound **5**



4 **5** IC_{50} = 150 nM

Reagents and conditions: (a) *n*-BuLi, THF, -78 °C, then H_20 at rt, 67%; (b) TFA, CH_2Cl_2, rt, 57%.

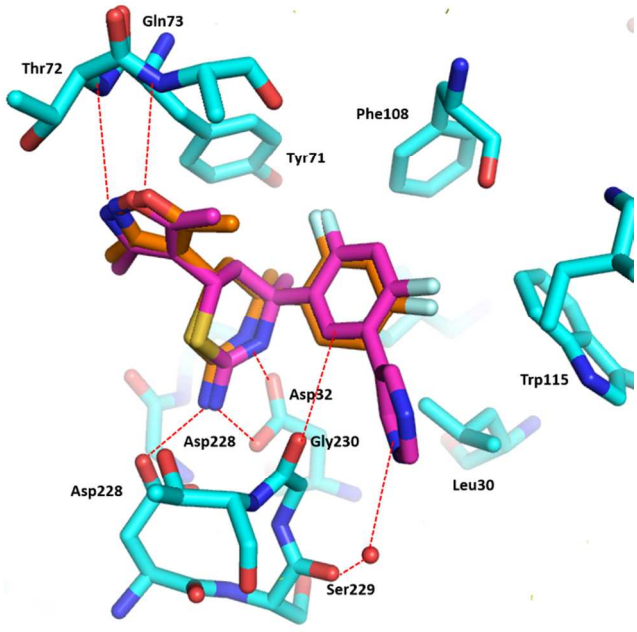


Figure 2. Overlay of co-crystal structures of **2** (magenta) and **5** (orange) bound with the BACE1 active site.

Despite its moderate potency, compound **5** produced a substantial pharmacodynamic effect, reducing brain Aβ by 62% at 3 hours post-dose when administered orally to young mice at 30 mg/kg. The total brain and plasma levels of **5** at 3 h post-dosing were 3.6 μM and 4.8 μM (brain/plasma: 0.75), respectively. In a similar experiment, biaryl aminothiazine **2** showed brain and plasma levels of 0.58 μM and 7.4 μM (brain/plasma: 0.08), respectively. Thus, the smaller analog **5** had a 6-fold improvement in brain exposure compared to **2**. Oral administration of compound **5** to rats also produced a dose-dependent reduction of Aβ42 and total Aβ ($Aβ_1$-x) in brain (Figure 3), and the levels of reduction were much more robust than those observed with the bi-aryl aminothiazine **2** (the dose response data of **2** not shown).

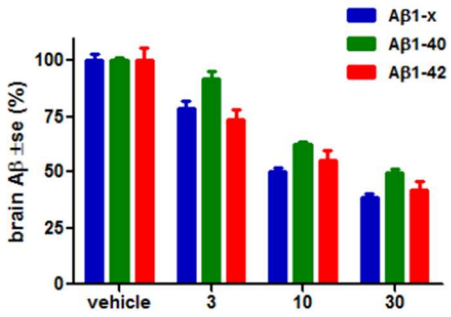


Figure 3. Dose-dependent inhibition of Aβ40, Aβ42 and $Aβ_{1-x}$ in the rat brain by compound 5 measured at 5 h after oral administration (n = 5 rats/dose). $p < 0.05$.

While compound **5** showed improved *in vivo* activity relative to **2**, its metabolic stability remained problematic. Compound 5 was relatively stable in rat and mouse liver microsomes, but unstable in human liver microsomes (78% and 72% and 24% of parent remained after a 10 minute incubation with mouse, rat and human liver microsomes, respectively, at 0.5 μM). Interestingly, the non-

fluorinated and monofluorinated analogs **6** and **7** exhibited superior stability in human liver microsomes, but their BACE1 inhibitory activity was diminished (Figure 4). Due to their reduced activity, both compounds afforded only marginal mouse brain Aβ reduction when dosed orally to mice at 30 mg/kg. The moderately active thiophene analog **8** displayed the worst metabolic stability profile.

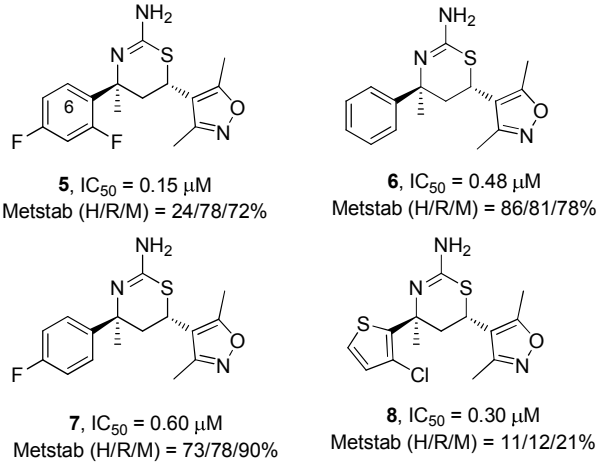


Figure 4. Analogs and metabolic stability (Metstab) in microsomes shown as % parent remaining after a 10 minute incubation at 0.5 μM; H = human, R = rat, M = mouse.

The activity enhancement by the 6-dimethylisoxazole group in the truncated series was clearly demonstrated by compound **6**, which is approximately 33-fold more potent than its C-6 unsubstituted counterpart **9** (IC_{50} = 22 μM from the literatute[12]). This magnitude of increase was even larger than that observed in the biaryl series (34-fold, Figure 5).[13] Overall, the trend was consistent in both series, but the degree of the enhancement depended on the substituent(s) of the phenyl group.

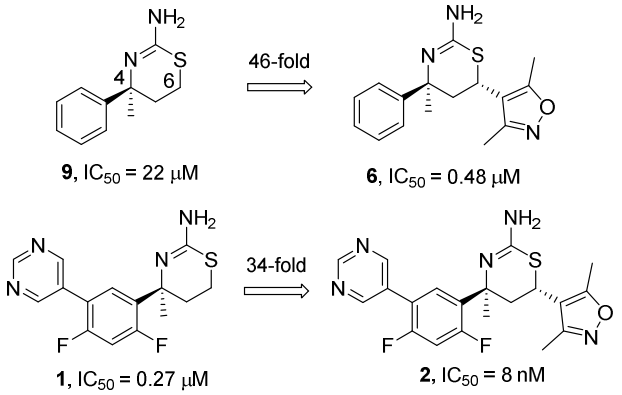
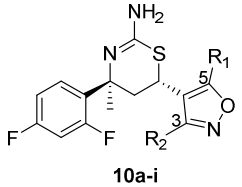


Figure 5. Activity enhancement by C-6 substitution.

A major metabolic pathway of compound **5** involved oxidation of the methyl group(s) attached to the isoxazole ring, and therefore, we attempted to block the metabolic soft spot(s) (Table 1). Replacement of the isoxazole methyl with ethyl (**10a**/**b**), phenyl (**10c**/**e**), substituted phenyl (**10d**/**f**), pyridyl (**10g**), and even the electron-withdrawing difluoromethyl (**10h**) exerted little influence on either potency or on metabolic stability. These substituents were positioned at the solvent interface and did not make any significant interactions with the BACE1 active site, thus resulting in comparable activity. Within this series, only the monomethyl substituted isoxazole analog **10i** exhibited improved metabolic stability across species. However, its modest *in vivo* activity prevented its further advancement.

Table 1. IC_{50} values of aminothiazines[a]



10a-i

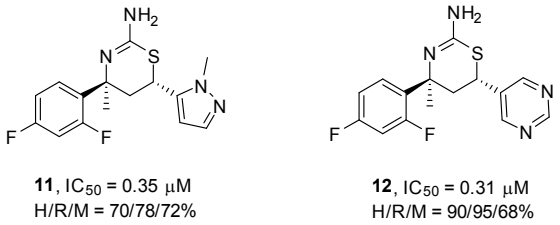
Cpd	R_1	R_2	IC_{50}	H/R/M

			(μM)	(%)[b]
10a	Me	Et	0.25	21/59/41
10b	Et	Et	0.42	35/67/59
10c	Me	Ph	0.16	38/13/12
10d	Me	*p*-F-Ph	0.32	24/35/23
10e	Ph	Me	0.23	39/20/6
10f	*p*-F-Ph	Me	0.41	45/88/53
10g	Me	3-Py	0.18	17/5/6
10h	CHF_2	Me	0.42	35/67/59
10i	H	Me	0.30	65/77/80

[a]All values are the mean of at least two separate assay determinations. [b]percent compound that remained after a 10 minute incubation with (H)uman, (R)at or (M)ouse liver microsomes at 0.5 μM.

Finally, we tackled our metabolic stability issue with **5** by replacing the entire dimethylisoxazole group with other heterocycles, an exercise that led to two analogs **11** and **12** with improved metabolic stability (Figure 6) and potency within 2-fold of isoxazole analog **5**. To understand if these heterocycles behaved in the same fashion as the dimethylisoxazole, we obtained a co-crystal structure of **12** bound with the BACE1 active site[16], and Figure 7 shows the overlay of co-crystal structures of both **5** (orange) and **12** (purple). Both molecules overlapped well, but the position of the flap backbone was different: the flap was more open with pyrimidine analog **12** (BACE1 in green) than with isoxazole analog **5** (BACE1 in cyan). As a result, the dimethylisoxazole was closer to the flap than the pyrimidine (dis-

tance from heteroatom of the heterocycle to the nitrogen of Thr72 or Glu 73: 3.4 Å for **5** versus 4.8 Å for **12**). While their potencies were close, the data suggested that the pyrimidine bound primarily in the hydrophobic pocket near the flap region, and the isoxazole made more contact with the flap as a result of the hydrogen bonds.



11, IC_{50} = 0.35 μM
H/R/M = 70/78/72%

12, IC_{50} = 0.31 μM
H/R/M = 90/95/68%

Figure 6.

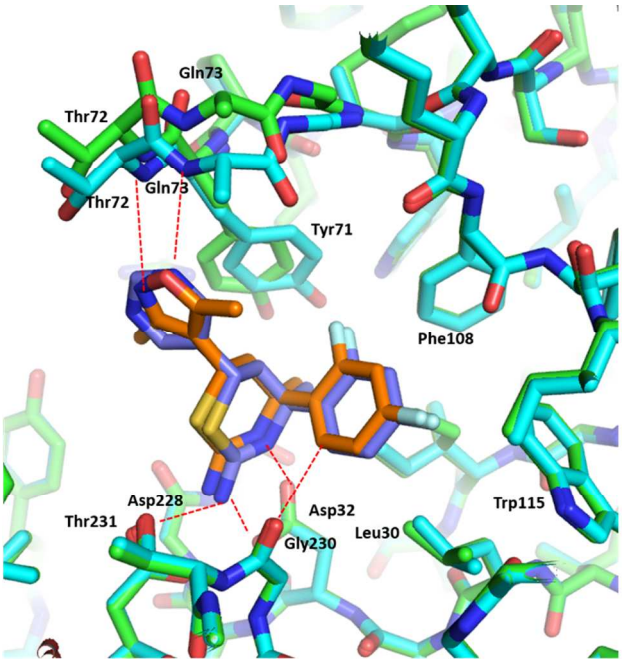


Figure 7. Overlay of co-crystal structures of **5** (orange) and **12** (purple) bound with the BACE1 active site. BACE1 in **5** is shown in green and BACE1 in **12** is shown in cyan.

Compounds **11** and **12** produced improved results in our mouse Aβ reduction screen versus compound **5**. Using a dose of 10 mg/kg delivered orally, **11** produced a 62% reduction, and **12** produced a 75% brain Aβ reduction. Both analogs demonstrated good plasma and brain drug levels (**11**, 4.3 μM, B/P = 1.3; **12**, 6.7 μM, B/P = 1.8). Because of its superior brain Aβ reduction in mice and good unbound fraction across species (37%, 48% and 42% unbound in human, rat and mouse plasma), compound **12** was advanced to rat dose-response studies. As shown in Figure 8, this compound elicited a dose-dependent reduction of Aβ40, Aβ42 and total Aβ1-x in brain. The exposure of **12** was also measured in rat plasma and brain samples (Table 2) to allow quantification of its PK/PD relationship. Analysis of the rat brain Aβ42 reduction versus the total plasma concentration curve (Figure 9) resulted in the estimation of a brain Aβ42 EC_{50} of 3.8 μM total plasma concentration or 1.8 μM free drug level in plasma (using rat plasma protein binding data), which was approximately 6-fold the cellular IC_{50} value of the compound (BACE1 IC_{50} = 0.31 μM).

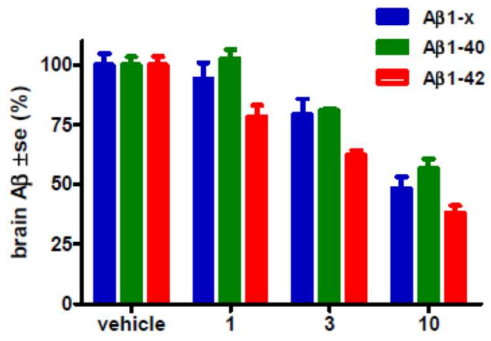
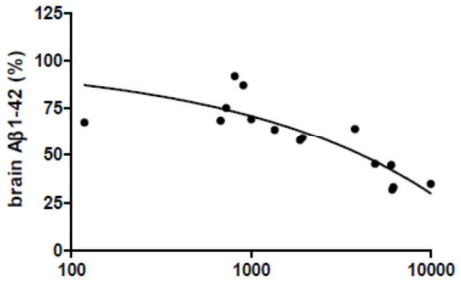


Figure 8. Dose-dependent reduction of Aβ40, Aβ42 and $Aβ_{1-x}$ in the brain by compound **12** measured at 5 h after oral administration (N = 5 rats/dose). $p < 0.05$.

Table 2. Brain, plasma concentration and brain/plasma ratio of **12**[a]

Dose (mg/kg)	Plasma Conc. μM (SD)	Brain Conc. μM (SD)	Brain/plasma ratio
1	0.82 (0.13)	2.3 (0.3)	2.8 (0.3)
3	1.8 (1.3)	2.8 (1.7)	1.8 (0.6)
10	6.6 (1.9)	12 (3)	1.8 (0.6)

[a]Compound dosed PO to female rats in PEG-400:EtOH:Tween 80 (84:15:1); plasma and brain collected 5h post-dose (N=5 rats/dose) for determination of exposure and brain Aβ levels. Data are mean values (N = 5) with SD in parentheses.



plasma concentration of 12

Figure 9. Brain Aβ42 reduction and plasma concentration curve of compound **12**.

Compound **12** was administered to rats as either an IV bolus (2 mg/kg) or PO (10 mg/kg) to provide rat IV/PO pharmacokinetic data. It exhibited low clearance (12 mL/min/kg, approximately 20% of rat hepatic blood flow) and a high volume of distribution (8.2 L/kg). The 10 mg/kg oral dose provided a

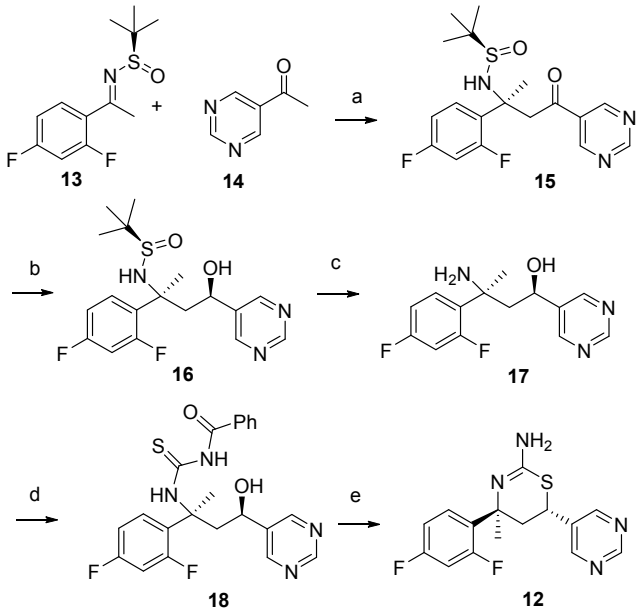
Cmax of 3.7 ± 0.6 μM at the 5 hour time point. The oral bioavailability based on the IV and PO AUC values was calculated to be more than 100%, potentially indicating nonlinear pharmacokinetics.

The cytochrome P450 enzyme inhibitory potential of **12** was determined using recombinant CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 enzymes in order to assess the potential likelihood of drug/drug interactions. Low levels of inhibition were observed across the major human P450 isozymes (IC_{50}'s > 20 μM) with the highest level of inhibition seen against CYP2C19 (IC_{50} = 9 μM).[17]

CHEMISTRY

Scheme 2 describes the synthesis of aminothiazine **12**. Stereoselective addition of the lithium enolate of methyl ketone **14** to *N*-*tert*-butanesulfinyl imine **13** gave the *N*-sulfinyl β-amino ketone **15**, which underwent stereoselective reduction with lithium tri-*tert*- butoxyaluminum hydride to give *syn*-1,3-*N*-sulfinyl-β-amino alcohol **16**.[18] Removal of the sulfinyl group led to the *syn*-1,3-amino alcohol **17**. Treatment of **17** with benzoyl isothiocyanate provided the benzoyl thiourea derivative **18**, which was converted to **12** under acidic conditions in moderate yield. Analogs **6-8**, **10a-10i**, and **11** were prepared following the same sequence.

Scheme 2. Synthesis of compound **12**



Reagents and conditions: (a) LDA, THF, -78 to -30 °C, 44%; (b) $LiAlH(O\text{-}tBu)_3$, ether, -78 to -30 °C, 93%; (c) 4N HCl in 1,4-dioxane, MeOH, 100%; (d) benzoyl isothiocyanate, Hunig's base, CH_2Cl_2, rt, 54%; (e) 1N H_2SO_4, 1,4-dioxane, 100 °C, 6 h, 42%.

CONCLUSIONS

we have developed a second approach to overcome the two major issues confronting biaryl aminothiazine **5**, which were poor brain penetration and metabolic instability. Truncation of the S3 substituent improved brain penetration, but a metabolic stability issue still remained. This issue was resolved through replacement of the 6-dimethylisoxazole with a pyrimidine ring. This work suggested that truncation of the S3 substituent was a viable approach to the discovery of orally bioavailable, brain-penetrant BACE1 inhibitors.

EXPERIMENTAL SECTION

General. Proton magnetic resonance (^{1}H NMR) spectra were recorded on either a Bruker Avance 400 or a JEOL Eclipse 500 spectrometer and are reported in ppm relative to the reference solvent of the sample in which they were run. HPLC and LC−MS analyses were conducted using a Shimadzu SCL-10A liquid chromatograph and a SPD UV−vis detector at 220 nm with the MS detection performed with either a Micromass Platform LC spectrometer or a Waters Micromass ZQ spectrometer. All flash column chromatography was performed on EM Science silica gel 60 (particle size of 40−60 μm). All reagents were purchased from commercial sources and used without further purification unless otherwise noted. All reactions were performed under an inert atmosphere. HPLC analyses were performed using the following conditions. All final compounds had an HPLC purity of ≥95% unless specifically mentioned.

HPLC Methods. Analytical HPLC analyses were carried out following methods A and B, and preparatory reverse-phase scale purifications were performed using methods C and D.

Method A. A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (solvent B) (t = 0 min, 10% B; t = 15 min, 100% B) was employed on a SunFire C18 3.5 μm 3.5 mm × 150 mm column. Flow rate was 0.5 mL/min, and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.

Method B. A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (solvent B) (t = 0 min, 10% B; t = 15 min, 100% B (20 min)) was employed on a XBridge Ph 3.5 μm 3.0 mm × 150 mm column. Flow rate was 0.5 mL/min, and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.

Method C. Column: Waters XBridge C18, 19 x 200 mm, 5-μm particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5-μm particles; Mobile Phase A: water with 20-mM ammonium acetate; Mobile Phase B: 95:5 methanol:water with 20-mM ammonium acetate; Gradient: 25-65% B over 40 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min.

Method D. Column: Waters Sunfire C18 OBD, 50 x 300 mm, 10-µm particles; Guard Column: Waters Sunfire C18 OBD, 50 x 50 mm, 10-µm particles; Mobile Phase A: 10:90 methanol:water with 0.1% trifluoroacetic acid; Mobile Phase B: 90:10 methanol:water with 0.1% trifluoroacetic acid; Gradient: 0-100% B over 30 minutes, then a 5-minute hold at 100% B; Flow: 150 mL/min.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-6-(3,5-dimethylisoxazol-4-yl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (**5**). Step A: To a solution of **4** (1 g, 1.936 mmol)[13] in THF (19.36 mL) at -78 °C was added dropwise *n*-BuLi (2.5M in hex) (3.10 mL, 7.75 mmol). The resulting mixture was stirred for 5 min. at -78 °C. The reaction mixture was then quenched by the addition of water (20 mL). The mixture was then allowed to come to rt at which time it was diluted with ethyl acetate (50 mL), washed with water (2 x 20 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel flash chromatography eluting with 0-35% ethyl acetate/Hexanes) gave tert-butyl ((4*S*,6*R*)-4-(2,4-difluorophenyl)-6-(3,5-dimethylisoxazol-4-yl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (581 mg, 67% yield) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 - 7.29 (m, 1H), 7.01 - 6.78 (m, 2H), 3.72 (dd, *J* = 13.1, 2.8 Hz, 1H), 2.87 (dd, *J* = 14.3, 2.8 Hz, 1H), 2.20 (s, 3H), 2.23 (1H, m), 1.76 (s, 3H), 1.53 (s, 9H). MS $(M+H)^+$: 438.1. Step B: To a solution of tert-butyl ((4*S*,6*S*)-4-(2,4-difluorophenyl)-6-(3,5-dimethylisoxazol-4-yl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-yl)carbamate (85 mg, 0.194 mmol) in DCM (3886 µl) was added TFA (388 µl, 5.04 mmol). The resulting mixture was stirred at rt for 3 h. The mixture was then concentrated *in vacuo*. Purification by flash chromatography (Silica, 12g, 0 - 15% MeOH/$CHCl_3$) gave a product which still contained an impurity. Further purification by preparative HPLC (Method D) followed by basification of the appropriate combined fractions with $NaHCO_3$ and extraction with ethyl acetate, drying ($MgSO_4$) of the organic extract and concentration gave **5** (39.6 mg, 57% yield) as a clear, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (td, *J* =9.1, 6.3 Hz, 1H), 7.04 - 6.83 (m, 2H), 3.85 (dd, *J* =12.9, 3.0 Hz, 1H), 2.90 (dd, *J* =14.3, 2.9 Hz, 1H), 2.30 (3H, s), 2.22 (3H, s), 2.14 (t, *J* =13.7 Hz, 1H), and 1.80 (3H, s). MS $(M+H)^+$: 338.1.

The absolute stereochemistry of this compound was confirmed by its co-crystal structure with BACE1 active site.

(4*S*,6*S*)-6-(3,5-Dimethylisoxazol-4-yl)-4-methyl-4-phenyl-5,6-dihydro-4H-1,3-thiazin-2-amine (**6**). To a solution of N-(((2*S*,4*R*)-4-(3,5-dimethylisoxazol-4-yl)-4-hydroxy-2-phenylbutan-2-yl)carbamothioyl)benzamide (117 mg, 0.276 mmol) (prepared following the sequence described in Ref. 13) in dioxane (921 µl) was added HCl (5M aqueous) (4420 µl, 22.10 mmol). The resulting mixture was brought to 90 °C and stirred for 1 h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until slightly basic. The mixture was then extracted with ethyl acetate (3 x 3 mL). The combined extracts were washed with water (2 mL), brine (2 mL), dried over MgSO4, filtered and concentrated in vacuo to give the crude oil. The crude material was purified via preparative HPLC (Method C) to give **6** (40 mg, 48%). ^{1}H NMR (500 MHz, DMSO-d6) δ 7.36 (d, *J* =4.3 Hz, 4H), 7.30 - 7.17 (m, 1H), 3.56 (dd, *J* =12.7, 2.9 Hz, 1H), 2.46 (dd, *J* =13.6, 2.9 Hz, 1H), 2.20 (s, 3H), 2.08 (s, 3H), 1.92 (s, 3H), 1.82 (t, *J* =13.3 Hz, 1H). MS (M+H)+: 302.1.

(4*S*,6*S*)-6-(3,5-Dimethylisoxazol-4-yl)-4-(4-fluorophenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (7). B To a solution of N-(((2*S*,4*R*)-4-(3,5-dimethylisoxazol-4-yl)-2-(4-fluorophenyl)-4-hydroxybutan-2-yl)carbamothioyl)benzamide (28 mg, 0.063 mmol) (prepared following the sequence described in Ref. 13) in dioxane (211 µl) was added HCl (5M aqueous) (1015 µl, 5.07 mmol). The resulting mixture was brought to 90 °C and stirred for 1 h. After coming to rt, the reaction mixture was brought to pH 8 by the addition of 1 N aqueous NaOH. The mixture was then extracted with ethyl acetate (3 x 5 mL). The combined extracts were washed with water (3 mL), brine (3 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The crude material was purified via preparative HPLC (Method C) to give 7 (4.6 mg, 0.014 mmol, 22.71 % yield) as a colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.48 - 7.40 (m, 2H), 7.32 (t, *J* =8.7 Hz, 2H), 3.93 - 3.79 (m, 1H), 2.96 - 2.86 (m, 1H), 2.50 - 2.43 (m, 1H), 2.28 (3H, s), 2.14 (3H, s) and 1.67 (3H, s). MS $(M+H)^+$: 320.1.

(4*S*,6*S*)-4-(3-Chlorothiophen-2-yl)-6-(3,5-dimethylisoxazol-4-yl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (**8**). To a solution of N-(((2*S*,4*R*)-2-(3-chlorothiophen-2-yl)-4-(3,5-dimethylisoxazol-4-yl)-4-hydroxybutan-2-yl)carbamothioyl)benzamide (460 mg, 0.991 mmol) (prepared following the sequence described in Ref. 13) in dioxane (10 mL) was added 6.0 M HCl (10 mL, 60.0 mmol). The mixture was stirred at 95 °C oil bath for 6 h. The mixture was concentrated, and saturated sodium bicarbonate was added. The aqueous layer was extracted with ethyl acetate, and the combined organic layes were dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated in vacuo, and the residue was purified via silica gel chromatography eluting with 0-100% ethyl acetate/hexanes to give **8** (120 mg, 35 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (d, *J* =5.3 Hz, 1H), 6.92 (d, *J* = 5.5 Hz, 1H), 4.03 - 4.03 (m, 1H), 3.99 (dd, *J* =12.8, 3.1 Hz, 1H), 3.12 (dd, *J* = 13.9, 3.1 Hz, 1H), 2.32 (s, 3H), 2.24 (s, 3H), 1.80 (dd, *J* = 13.8, 13.0 Hz, 1H), 1.69 (s, 3H). MS $(M+H)^+$: 342.05.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-6-(3-ethyl-5-methylisoxazol-4-yl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (**10a**). To a solution of N-(((2S,4R)-2-(2,4-difluorophenyl)-4-(3-ethyl-5-methylisoxazol-4-yl)-4-hydroxybutan-2-yl)carbamothioyl)benzamide (200 mg, 0.422 mmol) (prepared following the sequence described in Ref. 13) in dioxane (1408 µl) was added HCl (5M aqueous) (6758 µl, 33.8 mmol). The resulting mixture was brought to 90 °C and stirred for 3h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until slightly basic. The mixture was then extracted with ethyl acetate (3 x 3 mL). The combined extracts were washed with water (2 mL), brine (2 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to give the crude oil. The crude material was purified via preparative HPLC (Method C) to give **10a** (27 mg, 18%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.37 - 7.21 (m, 2H), 7.13 (td, *J* = 8.5, 2.6 Hz, 1H), 3.56 (dd, *J* = 13.1, 3.1 Hz, 1H), 2.57 (dd, *J* =13.9, 2.9 Hz, 1H), 2.45 (qd, *J* = 7.6, 4.4 Hz, 2H), 1.90 - 1.82 (m, 1H), 1.56 (s, 3H), 1.03 (t, *J* = 7.5 Hz, 3H). MS $(M+H)^+$: 352.0.

(4*S*,6*S*)-6-(3,5-Diethylisoxazol-4-yl)-4-(2,4-difluorophenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (**10b**). To a solution of N-(((2*S*,4*R*)-4-(3,5-diethylisoxazol-4-yl)-2-(2,4-difluorophenyl)-4 hydroxybutan-2-yl)carbamothioyl)benzamide (35 mg, 0.072 mmol) in dioxane (239 µl) was added HCl (5M aqueous) (1149 µl, 5.74 mmol). The resulting mixture was brought to 90 °C and stirred for 3 h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until slightly basic. The mixture was then extracted with ethyl acetate (3 x 3 mL). The combined extracts were washed with water (2 mL), brine (2 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to give the crude oil. The crude material was purified via preparative HPLC (Method C) to give **10b** (3.1 mg, 11% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 - 7.20 (m, 2H), 7.14 (td, *J* = 8.5, 2.6 Hz, 1H), 3.58 (d, *J* = 11.3 Hz, 1H), 2.66 (q, *J* = 7.6 Hz, 2H), 2.58 (d, *J* = 12.2 Hz, 1H), 2.48 (q, *J* = 7.6 Hz, 2H), 1.86 (m, 1H), 1.56 (s, 3H), 1.11 (3H,t, *J* = 7.6 Hz), 1.07 (3H, t, *J* = 7.6 Hz). MS $(M+H)^+$: 366.7.

(4S,6S)-4-(2,4-Difluorophenyl)-4-methyl-6-(5-methyl-3-phenylisoxazol-4-yl)-5,6-dihydro-4H-1,3-thiazin-2-amine (**10c**). To a solution of N-(((2*S*,4*R*)-2-(2,4-difluorophenyl)-4-hydroxy-4-(5-methyl-3-phenylisoxazol-4-yl)butan-2-yl)carbamothioyl)benzamide (118 mg, 0.226 mmol) in dioxane (1.2 ml) was added HCl (5M aqueous) (3.62 mL, 18.10 mmol). The resulting mixture was brought to 90 °C and stirred for 12 h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until slightly basic. The mixture was then extracted with ethyl acetate. The combined extracts were washed with water (2 mL), brine (2 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to give the crude oil which was purified by preparative HPLC (Method D) to afford **10c** TFA salt (48 mg, 0.093 mmol, 40.9 % yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.52 - 7.46 (m, 1H), 7.43 - 7.37 (m, 2H), 7.28 - 7.17 (m, 3H), 7.11 - 6.98 (m, 2H), 4.01 (dd, *J* =12.9, 2.8 Hz, 1H), 3.03 (dd, *J* = 14.7, 2.8 Hz, 1H), 2.65 (s, 3H), 2.62 - 2.54 (m, 1H), 1.84 (s, 3H). MS $(M+H)^+$: 400.2.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-6-(3-(4-fluorophenyl)-5-methylisoxazol-4-yl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (**10d**). To a solution of N-(((2*S*,4*R*)-2-(2,4-difluorophenyl)-4-(3-(4-fluorophenyl)-5-methylisoxazol-4-yl)-4-hydroxybutan-2-yl)carbamothioyl)benzamide (118 mg, 0.219 mmol) (prepared following the sequence described in Ref. 13) in dioxane (2.5 mL) was added HCl (5M aqueous) (3.50 mL, 17.50 mmol). The resulting mixture was brought to 90 °C and stirred for 16 h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until nearly neutral (17 mL). The mixture was extracted with ethyl acetate. The combined extracts were washed with water, brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The crude oil was purified using preparative HPLC (Method D) to afford **10d** TFA salt (56 mg, 47% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.29 - 7.19 (m, 3H), 7.17 - 7.10 (m, 2H), 7.10 - 6.99 (m, 2H), 3.95 (dd, *J* = 12.8, 2.7 Hz, 1H), 3.00 (dd, *J* = 14.7, 2.8 Hz, 1H), 2.62 (s, 3H), 2.55 (dd, *J* =14.6, 13.0 Hz, 1H), 1.82 (s, 3H). MS $(M+H)^+$: 418.1.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-4-methyl-6-(3-methyl-5-phenylisoxazol-4-yl)-5,6-dihydro-4H-1,3-thiazin-2-amine (**10e**). To a solution of N-(((2S,4R)-2-(2,4-difluorophenyl)-4-hydroxy-4-(3-methyl-5-phenylisoxazol-4-yl)butan-2-yl)carbamothioyl)benzamide (230 mg, 0.44 mmol) (prepared following the sequence described in Ref. 13) in dioxane (1.5 mL) was added HCl (5M aqueous) (7 mL, 35.3 mmol). The resulting mixture was brought to 90 °C and stirred for 16 h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until slightly basic. The mixture was then extracted with ethyl acetate (3 x 100 mL). The combined extracts were washed with water (25 mL), brine (25 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to give 290 mg of the crude oil. The crude material was purified via preparative HPLC (Method C) to give **10e** (44 mg, 24%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.32 - 7.24 (m, 1H), 7.18 (t, *J* = 7.6 Hz, 2H), 7.11 - 7.04 (m, 1H), 7.01 - 6.90 (m, 3H), 6.81 (t, *J* = 8.4 Hz, 1H), 3.62 (dd, *J* = 13.1, 2.4 Hz, 1H), 2.45 (d, *J* = 11.9 Hz, 1H), 2.18 (s, 3H), 1.75 (t, *J* = 13.4 Hz, 1H), 1.34 (s, 3H). MS $(M+H)^+$: 400.3.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-6-(5-(4-fluorophenyl)-3-methylisoxazol-4-yl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (**10f**). To a solution of N-(((2*S*,4*R*)-2-(2,4-difluorophenyl)-4-(5-(4-fluorophenyl)-3-methylisoxazol-4-yl)-4-hydroxybutan-2-yl)carbamothioyl)benzamide (473 mg, 0.877 mmol) (prepared following the sequence described in Ref. 13) in dioxane (2.9 mL) was added HCl (5M aqueous) (14 mL, 70.0 mmol). The resulting mixture was brought to 90 °C and stirred for 16h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until slightly basic. The mixture was then extracted with ethyl acetate (3 x 100 mL). The combined extracts were washed with water (25 mL), brine (25 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to give 290 mg of the crude oil. The crude material was purified via preparative HPLC (Method C) to give **10f** (51.8 mg, 14% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.42 - 7.27 (m, 5H), 7.25 - 7.17 (m, 1H), 7.11-6.94 (m, 1H), 3.77 (d, *J* =13.1 Hz, 1H), 2.67 (d, *J* =14.0 Hz, 1H), 2.40 (s, 3H), 1.95 (m, 1H), 1.56 (s., 3H). MS $(M+H)^+$: 418.3.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-4-methyl-6-(5-methyl-3-(pyridin-3-yl)isoxazol-4-yl)-5,6-dihydro-4H-1,3-thiazin-2-amine (**10g**). To a solution of N-(((2*S*,4*R*)-2-(2,4-difluorophenyl)-4-hydroxy-4-(5-methyl-3-(pyridin-3-yl)isoxazol-4-yl)butan-2-yl)carbamothioyl)benzamide (41 mg, 0.078 mmol) (prepared following the sequence described in Ref. 13) in dioxane (262 µl) was added HCl (5M aqueous) (1255 µl, 6.28 mmol). The resulting mixture was brought to 90 °C and stirred for 4 h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until slightly basic. The mixture was then extracted with ethyl acetate (3 x 100 mL). The combined extracts were washed with water (25 mL), brine (25 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The crude material was purified via preparative HPLC (Method C) to give **10g** (21 mg, 65%). ^{1}H NMR (500MHz, DMSO-d_6) δ 8.67 (d, *J* = 3.4 Hz, 1H), 8.45 (s, 1H), 7.58 (d, *J* = 7.3 Hz, 1H), 7.48 - 7.39 (m, 1H), 7.21 - 7.08 (m, 2H), 7.00 (t, *J* = 8.7 Hz, 1H), 3.62 (1H, m), 2.63 (d, *J* = 13.4 Hz, 1H), 1.87 - 1.78 (m, 1H), 1.52 (s, 3H). MS $(M+H)^+$: 401.0.

(4*S*,6*S*)-6-(5-(Difluoromethyl)-3-methylisoxazol-4-yl)-4-(2,4-difluorophenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (**10h**). To a solution of N-(((2*S*,4*R*)-4-(5-(difluoromethyl)-3-methylisoxazol-4-yl)-2-(2,4-difluorophenyl)-4-hydroxybutan-2-yl)carbamothioyl)benzamide (100 mg, 0.20 mmol) (prepared following the sequence described in Ref. 13) in dioxane (1.5 mL) was added HCl (5M aqueous) (3.2 mL, 16.2 mmol). The resulting mixture was brought to 90 °C and stirred for 16 h. The mixture was concentrated under high vacuum on the rotory evaporator. The residue was purified using preparative HPLC (Method D) to afford **10h** TFA salt (3.7 mg, 7.21 μmol, 4 % yield) as a clear residue. ^{1}H NMR (500 MHz, CD_3OD) δ 7.39 - 7.30 (m, 1H), 7.20 - 6.97 (m, 3H), 4.40 (dd, *J* =13.1, 3.4 Hz, 1H), 3.08 (dd, *J* =14.6, 3.4 Hz, 1H), 2.59 - 2.49 (m, 1H), 2.41 (s, 3H), 1.84 (s, 3H). MS $(M+H)^+$: 374.1.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-4-methyl-6-(3-methylisoxazol-4-yl)-5,6-dihydro-4H-1,3-thiazin-2-amine (**10i.**) To a solution of N-(((2*S*,4*R*)-2-(2,4-difluorophenyl)-4-hydroxy-4-(3-methylisoxazol-4-yl)butan-2-yl)carbamothioyl)benzamide (40 mg, 0.09 mmol) in dioxane (0.3 mL) was added HCl (5M aqueous) (1.4 mL, 7.2 mmol). The resulting mixture was brought to 90 °C and stirred for 16 h. The mixture was then allowed to come to rt and was quenched by the addition of aqueous 1N NaOH until slightly basic. The mixture was then extracted with ethyl acetate (3 x 100 mL). The combined extracts were washed with water (25 mL), brine (25 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified via preparative HPLC (Method C) to give **10i** (4.6 mg, 16 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (s, 1H), 7.36 - 7.05 (m, 3H), 3.65 (d, *J* = 12.8 Hz, 2H), 2.74 (br. s., 2H), 2.13 (s, 3H), 1.75 (t, *J* = 13.4 Hz, 1H), 1.55 (s, 3H). MS $(M+H)^+$: 324.3.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-4-methyl-6-(1-methyl-1H-pyrazol-5-yl)-5,6-dihydro-4H-1,3-thiazin-2-amine (**11**). To a solution of N-(((2*S*,4*R*)-2-(2,4-difluorophenyl)-4-hydroxy-4-(1-methyl-1H-pyrazol-5-yl)butan-2-yl)carbamothioyl)benzamide (134 mg, 0.30 mmol) (prepared following the sequence described in Ref. 13) in dioxane (1.5 mL) was added HCl (5M aqueous) (3.5 mL, 17.5 mmol).

The resulting mixture was sealed in a microwave vial and was brought to 90 °C and stirred for 16 h. The mixture was concentrated under high vacuum on the rotory evaporator. The residue was purified using preparatory HPLC (Method D) to afford **11** TFA salt (9.5 mg, 7 % yield) as a colorless oil. ^{1}H NMR (500 MHz, CD_3OD) δ 7.48 (dd, *J* = 2.1, 0.4 Hz, 1H), 7.43 - 7.34 (m, 1H), 7.21 - 7.10 (m, 2H), 6.50 (dd, *J* =2.0, 0.5 Hz, 1H), 4.41 (dd, *J* = 12.6, 3.1 Hz, 1H), 3.71 (s, 3H), 3.22 (dd, *J* =14.8, 3.2 Hz, 1H), 2.53 (dd, *J* = 14.8, 12.7 Hz, 1H), 1.87 (s, 3H). MS $(M+H)^+$: 323.0.

(4*S*,6*S*)-4-(2,4-Difluorophenyl)-4-methyl-6-(pyrimidin-5-yl)-5,6-dihydro-4H-1,3-thiazin-2-amine (**12**). To a solution of **18** (105 mg, 0.24 mmol) in dioxane (4 mL) was added 6.0 M HCl (4 mL, 24 mmol) at rt. The mixture was stirred at 95 °C for 1.5 h and at 55 °C for 18 h. The mixture was concentrated. The crude material was purified via preparative HPLC (Method C) to give **12** (32 mg, 42%). ^{1}H NMR (500 MHz, DMSO-d6) □ 9.10 (s, 1H), 8.85 (s, 2H), 7.94 - 7.60 (m, 1H), 7.18 - 6.81 (m, 2H), 4.79 (d, *J* = 8.9 Hz, 1H), 2.47 (br. s., 1H), 1.77 (d, *J* =11.3 Hz, 1H), 1.51 (s, 3H). MS $(M+H)^+$: 321.2. The absolute stereochemistry of this compound was confirmed by its co-crystal structure with BACE1 active site.

(*R*,*E*)-N-(1-(2,4-Difluorophenyl)ethylidene)-2-methylpropane-2-sulfinamide (**13**). To a solution of 1-(2,4-difluorophenyl)ethanone (5 g, 32.0 mmol) in THF (80 mL) was added *R*-(+)-*tert*-butansulfinamide (5.05 g, 41.6 mmol) and ethyl orthotitanate (14.5 mL, 70.5 mmol), and the resulting mixture was heated at 65 °C for 12 h. The reaction was quenched by the addition of water (200 mL), and the resulting mixture was filtered through Celite. The filtrate was then extracted with ethyl acetate (3 x 50 mL). The combined extracts were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by silica gel chromatography eluting with 30-70% ethyl acetate/hexanes to give **13** (3.1 g, 37 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 - 7.59 (m, 1H), 7.01 - 6.75 (m, 2H), 2.77 (d, *J* =2.7 Hz, 3H), 1.39 - 1.24 (s, 9H). MS $(M+H)^+$: 260.05.

(*R*)-N-((*S*)-2-(2,4-Difluorophenyl)-4-oxo-4-(pyrimidin-5-yl)butan-2-yl)-2-methylpropane-2-sulfinamide (**15**). To a solution of diisopropylamine (0.90 mL, 6.3 mmol) in THF (8 mL) was added a solution of 2.5 M *n*-BuLi in hexanes (2.8 mL, 6.9 mmol) at -78 °C under nitrogen. The mixture was stirred from -78 °C to 0 °C over 30 min. This LDA solution was slowly added to a solution of 1-(pyrimidin-5-yl)ethanone (733 mg, 6.00 mmol) in THF (10 mL) at -78 °C. After 30 min at -78 °C, a solution of **13** (778 mg, 3.0 mmol) in 1 mL of THF was added slowly. The mixture was stirred from -78 °C to -30 °C over 5 h. Water was added, and the solution was extracted with 2 x150 mL of ethyl acetate. The combined extracts were dried over sodium sulfate and then filtered. The filtrate was evaporated in vacuo, and the residue was purified via silica gel chromatography eluting with 0-100% ethyl acetate/hexanes to give **15** (500 mg, 44 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.31 (s, 1H), 9.14 (s, 2H), 7.56 (td, *J* =9.2, 6.4 Hz, 1H), 6.97 - 6.85 (m, 1H), 6.69 (ddd, *J* =12.9, 8.5, 2.6 Hz, 1H), 5.28 (s, 1H), 4.17 (dd, *J* = 18.6, 1.2 Hz, 1H), 3.86 (dd, *J* = 18.7, 2.8 Hz, 1H), 1.82 (s, 3H), 1.29 (s, 9H). MS $(M+H)^+$: 382.05.

(*R*)-N-((2*S*,4*R*)-2-(2,4-Difluorophenyl)-4-hydroxy-4-(pyrimidin-5-yl)butan-2-yl)-2-methylpropane-2-sulfinamide (**16**). To a solution of **15** (387 mg, 1.0 mmol) in ether (3 mL) was added 1.0 M lithium tri-tert-butoxyaluminum hydride in THF (2 mL, 2 mmol) at -78 °C under nitrogen. The mixture was stirred from -78 °C to -50 °C for 1 h. The reaction was quenched with $Na_2SO_4.10H_2O$, and 150 mL of ethyl acetate was added. After stirring at rt for 2 h, the solid was removed by filtration. The filtrate was concentrated, and the residue was purified via silica gel chromatography elutong with 0-10% methanol/ethyl acetate to give **16** (360 mg, 93 % yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.72 (s, 2H), 7.42 (td, *J* =9.0, 6.4 Hz, 1H), 6.88 - 6.80 (m, 1H), 6.72 (ddd, *J* = 12.4, 8.7, 2.7 Hz, 1H), 6.58 (dd, *J* =3.7, 1.1 Hz, 1H), 6.10 (s, 1H), 5.25 (d, *J* =10.4 Hz, 1H), 2.65 (dd, *J* = 14.8, 10.7 Hz, 1H), 2.11 (s, 3H), 1.93 (dd, *J* =14.8, 1.1 Hz, 1H), 1.22 (s, 9H). MS $(M+H)^+$: 384.16.

(1*R*,3*S*)-3-Amino-3-(2,4-difluorophenyl)-1-(pyrimidin-5-yl)butan-1-ol hydrochloride (**17**). To a solution of **16** (360 mg, 0.94 mmol) in MeOH (3 mL) was added 4.0 M HCl in dioxane (3 mL, 12.0 mmol) at rt. The mixture was stirred at rt for 1 h. The mixture was concentrated and azeotroped with toluene to give crude **17** hydrochloride (296 mg,100 % yield). This material was used for the next step without any purification. A small portion of this material was basified with saturated sodium bicarbonate and extracted with ethyl acetate to give **17** (free base). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.69 (s, 2H), 7.25 (td, *J* = 9.2, 6.3 Hz, 1H), 6.86 - 6.69 (m, 2H), 5.17 (dd, *J* = 10.4, 2.9 Hz, 1H), 2.08 - 1.86 (m, 2H), 1.73 (s, 3H). MS $(M+H)^+$: 280.05.

***N*-(((2*S*,4*R*)-2-(2,4-Difluorophenyl)-4-hydroxy-4-(pyrimidin-5-yl)butan-2-yl)carbamothioyl)benzamide** (**18**). To a solution of crude **17** hydrochloride (140 mg, 0.44 mmol) in DCM (3 mL) was added DIPEA (0.31 mL, 1.77 mmol) followed by benzoyl isothiocyanate (80 mg, 0.49 mmol) at rt. The mixture was stirred at rt for 1.5 h. The solvents were removed in vacuo, and the residue was was purified via silica gel chromatography 0-100% ethyl acetate/hexanes to give **18** (105 mg, 54 % yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.75 (s, 1H), 9.07 (s, 1H), 8.71 (s, 2H), 7.92 - 7.79 (m, 2H), 7.66 - 7.60 (m, 1H), 7.54 - 7.47 (m, 2H), 7.43 - 7.34 (m, 1H), 6.91 - 6.85 (m, 1H), 6.76 (ddd, *J* =12.2, 8.7, 2.6 Hz, 1H), 5.05 (d, *J* =7.8 Hz, 1H), 3.67 (d, *J* = 2.6 Hz, 1H), 2.93 (dd, *J* = 14.8, 9.6 Hz, 1H), 2.24 (3H, s), 2.21 (dd, *J* =14.8, 2.4 Hz, 1H). MS $(M+H)^+$: 443.05.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

X-ray data summary of compounds **5** and **12**, and methods for *in vitro*, *in vivo*, and pharmacokinetic assays.

Molecular formula strings (cvs).

Accession Codes

Structure coordinates of 5KR8 (**5**) and RKQF (**12**) have been deposited with the RSCB Protein Data Bank. Authors will release the atomic coordinates and experimental data upon article publication.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

TFA, trifluoroacetic acid; rt, room temperature; DCM, dichloromethane; Metstab, metabolic stability; CYP, cytochrome P450 enzymes; PO, oral administration; IV, intravenous administration; PK/PD, Pharmacokinetic/Pharmacodynamic; AUC, area under curve; B/P, brain/plasma concentration ratio.

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(14) Structure coordinates have been deposited with the RSCB Protein Data Bank (PDB ID 5KR8). For details on crystallization and structure determination see the Supplemental Section.

(15) For an example of applying hydrogen-bond interactions involving 3,5-dimethylisoxazole in drug discovery, see: Hewings, D. S.; Wang, M.; Philpott, M.; Fedorov, O; Uttarkar, S.; Filippakopoulos, P; Picaud, S.; Vuppusetty, C.; Marsden, B.; Knapp, S.; Conway, S. J.; Heightman, T. D. . 3,5-Dimethylisoxazoles act as acetyl-lysine-mimetic bromodomain ligands *J. Med. Chem*. **2011**, *54*, 6761-6770.

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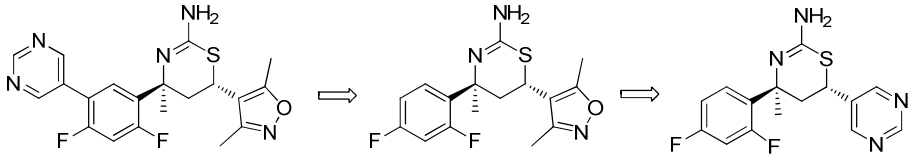
Table of Contents Graphic



2 IC_{50} = 8 nM **5** IC_{50} = 0.15 μM **12** IC_{50} = 0.31 μM