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Preparation and biological evaluation of conformationally constrained BACE1 inhibitors

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ABSTRACT

The BACE1 enzyme is a key target for Alzheimer's disease. During our BACE1 research efforts, fragment screening revealed that bicyclic thiazine **3** had low millimolar activity against BACE1. Analysis of the co-crystal structure of **3** suggested that potency could be increased through extension toward the S3 pocket and through conformational constraint of the thiazine core. Pursuit of S3-binding groups produced low micromolar inhibitor **6**, which informed the S3-design for constrained analogs **7** and **8**, themselves prepared via independent, multi-step synthetic routes. Biological characterization of BACE inhibitors **6**–**8** is described.

1. Introduction

Alzheimer's disease is a neurodegenerative disorder characterized by progressive memory loss ultimately leading to dementia. A key hallmark of the disease is the abnormal extracellular accumulation of Aβ in the brain as soluble oligomers or insoluble plaques.[1–5] Owing to its critical role in the production of Aβ, BACE1 has been aggressively pursued as a key target for development of a potential Alzheimer's disease therapy.[6–8] We have previously reported on the fragment-based discovery of LY2811376 (**1**, Fig. 1), the first molecule reported to demonstrate robust reduction of human CSF Aβ in a Phase I clinical trial.[9] More recently, we reported the discovery of LY2886721 (**2**), a potent BACE1 inhibitor that reached Phase 2 clinical trials in early Alzheimer's disease.[10] As part of our Alzheimer's disease program, we also targeted topologically complex, polycyclic aminothiazines as BACE1 inhibitors to test conformational constraint and S3-pocket binding hypotheses. The successful testing of these medicinal chemistry hypotheses through multi-step organic synthesis and the biological characterization of the resulting BACE inhibitors is described herein.

2. Results and discussion

2.1. General design considerations

During our fragment-based discovery efforts toward BACE1 inhibitors, we identified bicyclic aminothiazine fragment (±)-**3** (Fig. 1), which weakly inhibited BACE1 in a high concentration recombinant BACE1 enzyme assay. Crystallization of (±)-**3** with BACE1 revealed a binding mode in which the aminothiazine engaged in a hydrogen bonding network with the catalytic aspartic acids, with the fused cyclohexyl ring projecting into the S1 pocket (Fig. 2). Based on the X-ray structural observations and computational docking studies, we hypothesized that the potency and efficiency of this fragment could be increased through two key structural changes: conformational constraint to reinforce the observed binding mode, and extension of suitable functionality toward the S3 pocket of the enzyme.

Thiazine **4** was initially targeted to test these hypotheses. Although a conformational analysis revealed that the bicyclic

Abbreviations: MBP, maltose binding protein; mcaFRET, (7-methoxycoumarin-4-yl)acetyl modified fluorescence resonance energy transfer; MDCK, Madin-Darby canine epithelial cells; NT, not tested; PDAPP, transgenic mice overexpressing mutant human amyloid precursor protein V717F.

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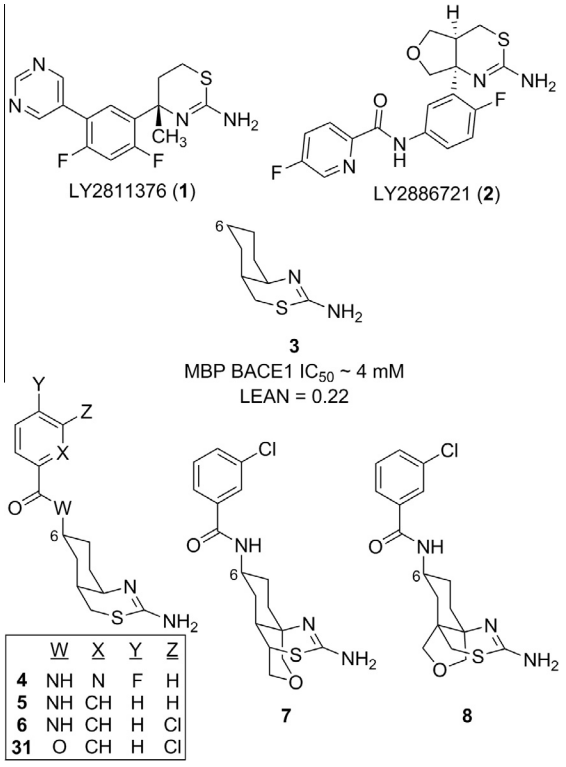


	W	X	Y	Z
4	NH	N	F	H
5	NH	CH	H	H
6	NH	CH	H	Cl
31	O	CH	H	Cl

Figure 1. Aminothiazine BACE1 inhibitors.

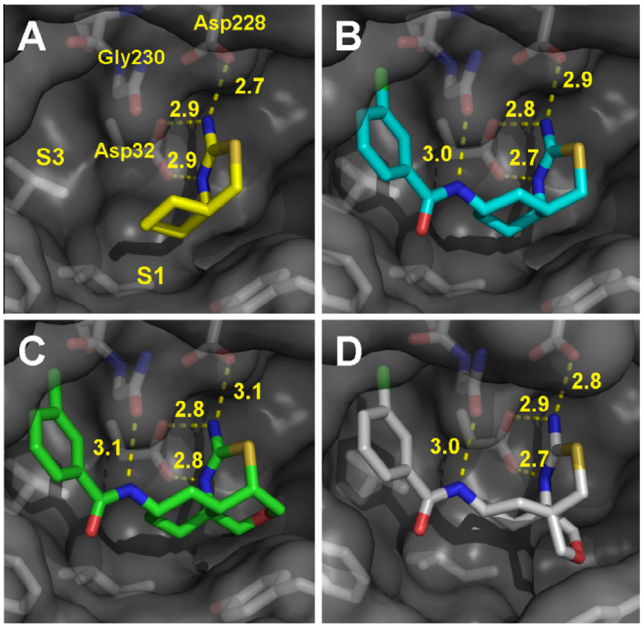


Figure 2. X-ray crystal structures of BACE1 with **3** (A), **6** (B), **7** (C), and **8** (D). Oxygen, nitrogen, chlorine and sulfur atoms are colored red, blue, green and yellow, respectively. Hydrogen bond distances (in Å) are indicated in yellow.

thiazines **3** and **4** appeared to bind in their low energy conformations, it was hypothesized that additional constraints could essentially lock the compounds into their binding conformations, providing meaningful increases in potency.[11]

2.2. Initial SAR

Compound **4**, which, like **2**, contains a fluoropyridine S3 binding group, was targeted to test the S3 binding hypothesis while syntheses of more complex constrained scaffolds were pursued. This molecule exhibited an mcaFRET BACE1 IC_{50} of 37.5 μM, a ~100-fold increase in potency compared to fragment **3**. Surprisingly, however, unsubstituted benzamide **5** displayed similar potency and improved efficiency compared to **4**, prompting re-evaluation of the substitution of the aromatic amide ring. This led to the discovery of **6**, which displayed a BACE1 IC_{50} value of 1.83 μM (LEAN = 0.27).[12] This represented a >1500-fold increase in potency compared to fragment **3**, a significant increase in ligand efficiency, and resulted in BACE1 potency within ~10-fold of LY2811376 (**1**), which had already demonstrated robust reduction of human CSF Aβ.[9] The BACE1 potency of **6** bolstered our interest in the constrained scaffolds to increase potency.

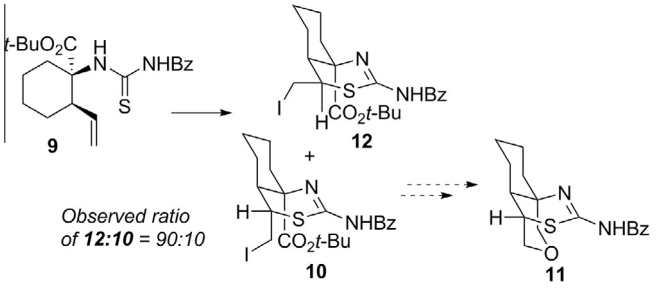
An X-ray crystal structure of **6** bound in the BACE1 active site indicated that the binding mode of fragment **3** was conserved, with the amide extending toward the S3 pocket (Fig. 2). The binding mode of amide **6** indicated that the core aromatic group preferred to bind at the entrance to the S3 pocket with the chloro substituent extending into the pocket. Space appeared to be available in the BACE1 active site to constrain the bicyclic aminothiazine via three-atom fused or bridged ring systems. Furan and pyran rings were selected as constraints over the corresponding all-carbon rings owing to the presumed metabolic and physicochemical property benefits afforded by the oxygen-containing constraints. Based on these X-ray observations and the SAR data for **4**, **5**, and **6**, we targeted *meta*-chloro analogs **7** and **8** (Fig. 1).

2.3. Chemistry

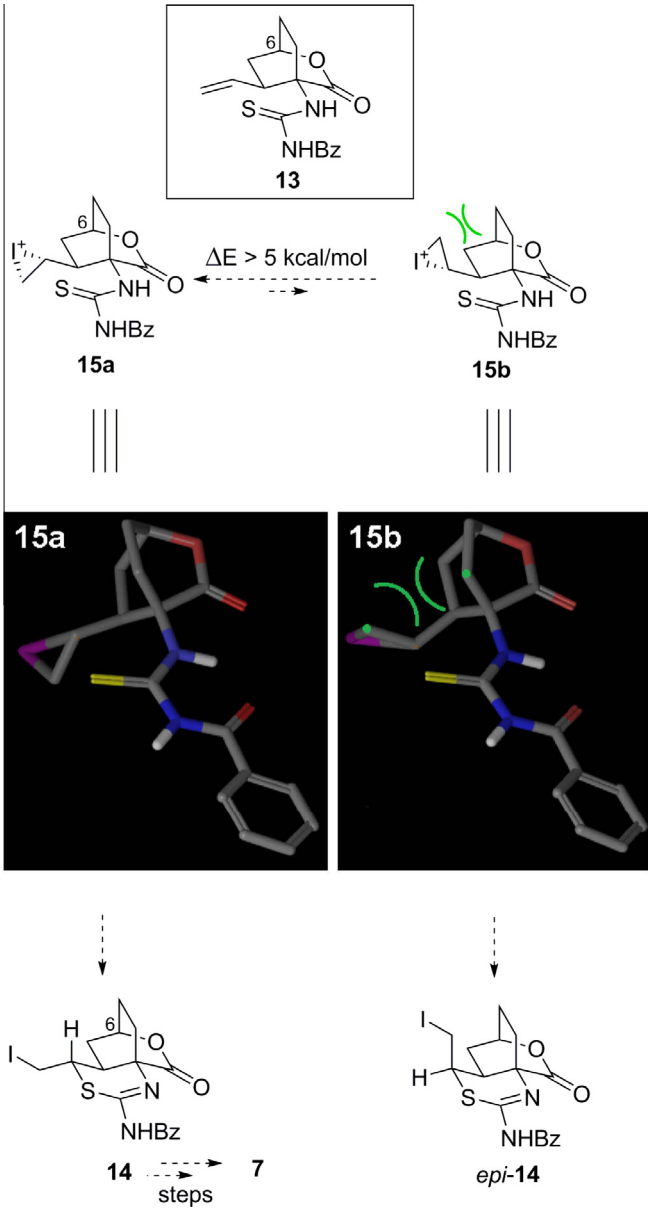
2.3.1. Model tricyclic system

In parallel with the aforementioned SAR, the chemistry toward tricycle **7** was piloted on model thiourea **9**,[13] lacking the amide substituent. The two key steps in the proposed reaction sequence were an iodocyclization reaction (conversion of **9**–**10**) and a reductive etherification (conversion of **10**–**11**) (Scheme 1). In order to generate **11** via this sequence, the diastereoselectivity of the iodocyclization would need to favor **10** over **12**. Thiourea **9** was prepared to explore the iodocyclization/etherification sequence.[14] Upon treatment of **9** with I_2 in CH_2Cl_2, iodocyclization proceeded smoothly, affording a mixture of isomers in 76% yield. However, undesired isomer **12** was the major product with a diastereomeric ratio of roughly 90:10.[15] Given the undesired selectivity exhibited by **9**, we elected to target a different cyclization substrate to access the key tricyclic framework present in **7**.

We reasoned that the preference for isomer **12** in the cyclization of **9** may have been driven by the development of a steric interaction between the ester and the iodomethyl substituent in the transition state leading to **10**. In our revised synthetic approach, we posited that constraining the ester via bridged lactone **13** (Scheme 2) would reverse the stereochemical preference of the cyclization in favor of **14**. This was supported by computational modeling, which suggested that conformer **15a** (leading to



Scheme 1. Initial model system for iodocyclization.



Scheme 2. Putative conformations leading to **14** (**15a**) and *epi*-**14** (**15b**). Note the strain shown in green disfavoring **15b**.

14) was significantly lower in energy than conformer **15b** (leading to *epi*-**14**) owing to a steric interaction (shown in green) in **15b** between the iodonium species and the carbon bridge.[16] We expected that this energy difference would be reflected in the transition states arising from **15a** and **15b**, thereby favoring **14**. Furthermore, this constrained system would contain functionality at C(6), enabling installation of the amide side chain late in the synthetic sequence, which would facilitate additional SAR.

2.3.2. Synthesis of 7

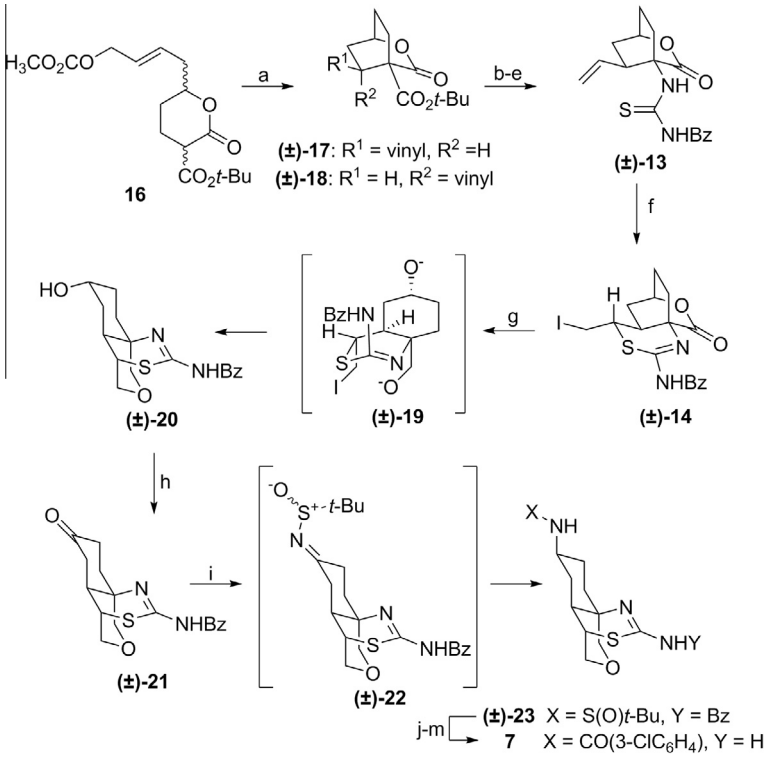
Toward the synthesis of **7** (Scheme 3), allyl carbonate **16**[14] was treated with *n*-BuLi followed by $Pd(PPh_3)_4$ to induce an allylic substitution, furnishing (±)-**17** in 31% isolated yield, along with 17% of the undesired epimer (±)-**18**. Cleavage of the *t*-butyl ester and subsequent Curtius rearrangement afforded the corresponding benzyl carbamate. Removal of the Cbz group and acylation with benzoyl isothiocyanate then afforded thiourea (±)-**13** as the key iodocyclization substrate. Treatment of (±)-**13** with I_2 in dichloromethane afforded (±)-**14** as the sole product in 75% yield, supporting our hypothesis that constraining the ester via a bicyclic lactone would alter the stereochemical outcome of the iodocyclization.[15] After extensive experimentation, it was found that one-pot reductive ring opening of lactone (±)-**14** and in situ cyclization of the resulting putative alkoxide (±)-**19** afforded (±)-**20** in 19% yield. Alcohol (±)-**20** was then oxidized to ketone (±)-**21**, which was converted to sulfinyl imine (±)-**22** via Ti(IV)-promoted condensation with *t*-butyl sulfinamide. In situ reduction of (±)-**22** with sodium borohydride proceeded exclusively through axial approach on the conformationally locked system to furnish (±)-**23** as the sole observed diastereomer. Finally, deprotection of (±)-**23** followed by acylation to install the 3-chlorobenzoyl group, chiral separation, and then removal of the benzoyl protecting group produced aminothiazine **7**. The synthesis of **7** included 18 steps and proceeded in 0.1% overall yield from commercially available materials.

2.3.3. Synthesis of 8

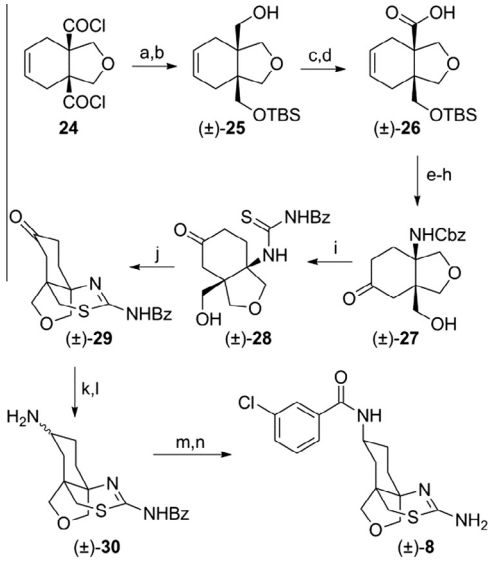
Constrained tricycle (±)-**8** was prepared via a 21-step linear sequence for study alongside **7** (Scheme 4).[14] Acid chloride **24**[17] was reduced to the corresponding diol and mono-silylated to yield (±)-**25**. Two-step oxidation furnished acid (±)-**26**, which was subjected to a Curtius rearrangement and hydroboration/oxidation. Oxidation of the resulting crude alcohol, desilylation, and separation of regioisomers then provided ketone (±)-**27**. Hydrogenolysis of the benzyl carbamate was followed by treatment with benzyl isocyanate to provide urea (±)-**28**. Activation of the alcohol with Ghosez's reagent[18] triggered cyclization to yield the thiazine (±)-**29**. To convert the ketone to the amine, a similar protocol was employed to that used for the conversion of (±)-**21** to (±)-**23**. However, this reduction produced only a 3:2 mixture of diastereomers. Deprotection of the resulting sulfonamide yielded (±)-**30**, which was acylated and deprotected to give the target compound (±)-**8**.

2.4. In vitro and in vivo evaluation

The successful delivery of aminothiazines **7** and (±)-**8** enabled us to test the hypothesis that conformational constraint would increase potency. Tricycle **7** displayed a BACE1 IC_{50} of 2.25 μM, similar to that of the unconstrained molecule **6**, while (±)-**8** demonstrated a BACE1 IC_{50} of 36.1 μM, which was considerably less potent than **6** (Table 1). The crystal structure of **7** complexed to BACE1 suggested that the conformational constraint imposed by the bridged pyran system had indeed reinforced the bound conformation of **6** (compare Fig. 2, panels B and C). However, this conformational constraint did not translate to greater in vitro BACE1 potency. Gas phase conformational analysis of **6** estimated that the alternate boat conformation of the cyclohexyl ring to be approximately 1.3 kcal/mol above the favored chair, which would result in a small but significant population; however, the anticipated entropic gain in binding free energy was not realized when this conformation was eliminated by the pyran ring constraint in **7**. On the other hand, the X-ray structure of **8** suggests that substitution on the bridgehead position to install the constraint, is not well tolerated in the binding pocket as it evidentially projects too far into the mobile flap region, resulting in decreased potency. Further biochemical characterization revealed that **6** and **7** did not display significant cathepsin D inhibition up to concentrations of 100 and 300 μM, respectively. However, neither compound displayed selectivity against BACE2.[19] Compounds **6** and **7** also possessed low mouse microsomal metabolism. Given the single-digit micromolar potency, low microsomal mouse metabolism, and the level of synthetic investment in the initial SAR, we elected to use **6** and **7** as tool compounds for in vivo assessment. Both molecules were administered subcutaneously at 100 mg/kg to young female



Scheme 3. Synthesis of **7**. Reagents and conditions: (a) *n*-BuLi, toluene, −78 °C, then $Pd(PPh_3)_4$, −78 °C to rt, 31% (±)-**17** and 17% (±)-**18**; (b) (±)-**17**, TFA, CH_2Cl_2, 96%; (c) DPPA, BnOH, Et_3N, toluene, 79%; (d) $Pd(OAc)_2$, Et_3SiH, Et_3N, CH_2Cl_2, rt, 77%; (e) benzoyl isothiocyanate, THF, rt, 91%; (f) I_2, CH_2Cl_2, 0 °C–rt, 75%; (g) $LiBH_4$, THF, H_2O, 19%; (h) TPAP, NMO, CH_2Cl_2, 85%; (i) (±)-*tert*-butyl sulfinamide, $Ti(OEt)_4$, THF, 65 °C, then $NaBH_4$, MeOH, −30 °C, 99%; (j) HCl, Et_2O, 91%; (k) 3-chlorobenzoic acid, HOBt, EDCI, DIEA, THF, 91%; (l) chiral SFC, 43%; (m) $MeONH_2$–HCl, pyridine, EtOH, 50 °C, 93%.



Scheme 4. Synthesis of **8**. Reagents and conditions: (a) $LiAlH_4$, THF, 80 °C, 92%; (b) NaH, THF, then TBSCl, rt, 83%; (c) PCC, CH_2Cl_2, rt, 78%; (d) $NaClO_2$, 2-methyl-2-butene, *t*-BuOH, THF, NaH_2PO_4(aq), 0 °C–rt, 100%; (e) BnOH, DIEA, toluene; DPPA, 110 °C, 77%; (f) BH_3–$S(CH_3)_2$, THF, then NaOH(aq), H_2O_2 (aq); (g) DMP, CH_2Cl_2, rt, 88% (2 steps); (h) TBAF, THF, 50%; (i) H_2, Pd/C, EtOH, EtOAc, then benzoyl isothiocyanate, 76%; (j) 1-chloro-*N*,*N*,2-trimethylpropenylamine, CH_2Cl_2, 91%; (k) (±)-*tert*-butyl sulfinamide, $Ti(OEt)_4$, THF, 65 °C, then $NaBH_4$, MeOH, 0 °C–rt, 52%, 3:2 dr; (l) HCl, Et_2O, CH_2Cl_2, 82%; (m) 3-chlorobenzoic acid, HOBt, EDCI, DIEA, THF, 24%; (n) $MeONH_2$–HCl, pyridine, EtOH, 50 °C, 94%.

Table 1
Physicochemical and selectivity data for BACE1

	6·HCl	**7**·HCl	(±)-**8**·HCl	**31**·TFA
MW	324	366	366	325
*c*log*P*	2.6	2.2	2.2	3.5
PSA (Å^2)	68	77	77	65
pK_a	9.7	9.0	8.2	—
BACE1 IC_{50} (μM)	1.83	2.25	36.1	0.761
LEAN	0.27	0.24	0.19	0.29
BACE2 IC_{50} (μM)	0.497	1.34	22.7	0.584
Cathepsin D IC_{50} (μM)	>100	>300	277	>100
Pgp efflux ratio	10	8.5	NT	2.7
Permeability (10^{-6} cm/s)	2.9	2.2	NT	7.2

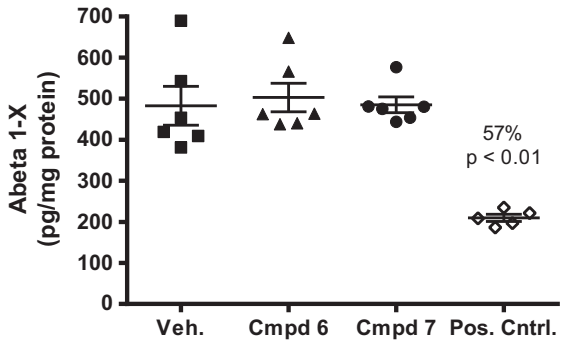


Figure 3. Brain Aβ concentrations in PDAPP mice after subcutaneous injection of **6** and **7**.

PDAPP (V717F) transgenic mice[20,21] and total Aβ was measured in brain cortex samples by sandwich ELISAs.[9,22] Unlike other molecules with low micromolar BACE1 potency that we had tested in PDAPP mice, neither **6** nor **7** produced significant changes in Aβ concentrations in brain (Fig. 3). Concentrations of compounds **6** and **7** were measured at 9.3 and 6.7 μM in plasma, but 0.75 and

0.61 μM in brain, respectively. The lack of reduction in brain Aβ levels was consistent with the low brain exposure, with total brain concentrations of each compound representing less than 0.3-fold the respective in vitro IC_{50} value. The low in vivo brain penetration for these compounds is consistent with the low permeability and high P-gp mediated efflux subsequently observed in MDCK cells (Table 1).[23] Additional SAR within the bicyclic scaffold demonstrated that replacement of the amide with an ester linkage (**31**, Fig. 1) eliminated P-gp efflux and increased permeability (Table 1). These data suggest that the amide linkage contributes to the P-gp efflux and low permeability observed with **6** and **7**.

3. Conclusions

After aminothiazine **3** was identified as a promising fragment starting point through high concentration in vitro testing and X-ray fragment soaking experiments, a structure-based design approach was pursued to optimize this fragment. This strategy was enabled by the execution of challenging synthetic chemistry which, in the case of constrained molecule **7**, required a second generation approach to produce the final molecule. Although compounds **6** and **7** did not achieve significant Aβ reduction in PDAPP mice owing to low brain exposure, the successful synthesis of the compounds enabled key SAR hypotheses to be tested, revealing that the potency of this series of BACE inhibitors was increased by >1500-fold through S3 binding, but was not further enhanced through conformational constraint. This work exemplifies the continued importance of synthetic organic chemistry in the pursuit of increasingly difficult drug discovery targets.[24]

4. Experimental section

4.1. Chemistry

4.1.1. (±)-*tert*-Butyl 3-oxo-5-*exo*-vinyl-2-oxabicyclo[2.2.2]-octane-4-carboxylate ((±)-17)

A −78 °C solution of **16** (7.457 g, 22.7 mmol) in dry toluene (42 mL) was treated dropwise with a solution of *n*-butyl lithium in hexanes (1.6 M, 14.9 mL, 23.9 mmol) and the reaction was stirred at −78 °C for 2 min under nitrogen and was then treated with tetrakis(triphenylphosphine)palladium (1.31 g, 1.14 mmol), and the reaction was warmed to room temperature and stirred overnight. The reaction was quenched with 1.0 N aqueous HCl, diluted with water, and extracted three times with EtOAc. The combined organic layers were dried (Na_2SO_4) and the solvent was removed to afford crude product that was purified on silica gel with a step gradient of 10% then 20% EtOAc in hexanes to afford (±)-**17** (1.778 g, 31% yield) and (±)-**18** (0.969 g, 17% yield).

(±)-**17**: R_f = 0.25 (3:1 hexanes/EtOAc, $KMnO_4$ stain) ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78–5.72 (m, 1H), 5.21 (dt, *J* = 5.7, 1.3 Hz, 1H), 5.18–5.17 (m, 1H), 4.69–4.66 (m, 1H), 3.21–3.19 (m, 1H), 2.32–2.29 (m, 1H), 2.16–2.09 (m, 3H), 1.74–1.68 (m, 1H), 1.61–1.58 (m, 1H), 1.45 (s, 9H). LCMS (ESI^+) (*m*/*z*) 197 $[M+2H-t\text{-}Bu]^+$.

(±)-**18**: R_f = 0.15 (3:1 hexanes/EtOAc, $KMnO_4$ stain) ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.79–5.73 (m, 1H), 5.03–5.01 (m, 1H), 4.99–4.98 (m, 1H), 4.67–4.64 (m, 1H), 2.95–2.90 (m, 1H), 2.25–2.17 (m, 1H), 2.08–2.04 (m, 3H), 1.89–1.84 (m, 1H), 1.79–1.76 (m, 1H), 1.43 (s, 9H). LCMS (ESI^+) (*m*/*z*) 197 $[M+2H-t\text{-}Bu]^+$.

4.1.2. (±)-*N*-((3-Oxo-5-*exo*-vinyl-2-oxabicyclo[2.2.2]octan-4-yl)carbamothioyl)benzamide ((±)-13)

A solution of (±)-**17** (1.82 g, 7.21 mmol) in CH_2Cl_2 (40 mL) was treated with TFA (20 mL) and stirred at room temperature for 1 h. The solvent was removed, and the residue was diluted with water and EtOAc. The aqueous layer was extracted with EtOAc (3×). The combined organic layers were dried (Na_2SO_4) and the solvent was removed to afford (±)-*tert*-butyl 3-oxo-5-*exo*-vinyl-2-oxabicyclo[2.2.2]octane-4-carboxylate (1.36 g, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.87–5.81 (m, 1H), 5.28 (s, 1H), 5.26–5.24 (m, 1H), 4.79–4.77 (m, 1H), 3.24–3.20 (m, 1H), 2.40–2.34 (m, 2H), 2.16–2.11 (m, 2H), 1.83–1.75 (m, 1H), 1.68 (ddd, *J* = 14.4, 4.4, 1.3 Hz, 1H). LCMS (ESI^-) (*m*/*z*) 195 $[M-H]^-$.

A room temperature solution of the product from the previous step (1.36 g, 6.93 mmol), benzyl alcohol (3.00 g, 27.7 mmol) and triethylamine (2.81 g, 27.7 mmol) in toluene (60 mL) was treated with diphenylphosphonic azide (2.29 g, 8.32 mmol), then the reaction was heated to reflux for 4 h with stirring under nitrogen. The reaction was cooled to room temperature, and then diluted with water and EtOAc. The organic layer was washed with 1 N aqueous HCl and brine. The aqueous layer was extracted two more times with EtOAc. The combined organic layers were dried (Na_2SO_4) and the solvent was removed to afford crude product that was purified on silica gel with a 0–100% gradient of EtOAc in hexanes to afford (±)-4-amino-5-*exo*-vinyl-2-oxabicyclo[2.2.2]octan-3-one (1.66 g, 79% yield). R_f = 0.30 (1:1 hexanes/EtOAc, $KMnO_4$ stain to visualize). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.27 (m, 5H), 5.98–5.95 (m, 1H), 5.21–5.12 (m, 3H), 5.08 (s, 2H), 4.71–4.68 (m, 1H), 3.06–2.96 (m, 1H), 2.60–2.48 (m, 1H), 2.45–2.36 (m, 1H), 2.20–1.96 (m, 2H), 1.89–1.81 (m, 1H), 1.69–1.64 (m, 1H). LCMS (ESI^+) (*m*/*z*) 302 $[M+H]^+$.

A room temperature mixture of triethylsilane (1.60 g, 13.77 mmol) and triethylamine (84 mg, 826 μmol) in dry CH_2Cl_2 (40 mL) was treated with $Pd(OAc)_2$ (93 mg, 413 μmol) and stirred under nitrogen for 5 min and the dark mixture was then treated dropwise over 5 min with a solution of the product from the previous step (1.66 g, 5.51 mmol) in CH_2Cl_2 (40 mL) and the reaction was stirred at room temperature for 7 h under nitrogen. The solvent was removed, and the crude product was purified on silica gel with a 1–10% (80% MeOH/20% isopropylamine) in CH_2Cl_2 gradient, and the mixed fractions were purified with a 10 g SCX column using 2:1 CH_2Cl_2/MeOH and then 2:1 (CH_2Cl_2/7 N NH_3) in MeOH to afford (±)-4-amino-5-*exo*-vinyl-2-oxabicyclo[2.2.2]octan-3-one (0.714 g, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.97–5.88 (m, 1H), 5.27 (dt, *J* = 10.0, 1.1 Hz, 1H), 5.19 (dt, *J* = 17.0, 1.1 Hz, 1H), 4.67–4.64 (m, 1H), 2.47–2.46 (m, 1H), 2.38–2.34 (m, 1H), 2.12–2.06 (m, 2H), 1.86–1.80 (m, 1H), 1.66–1.61 (m, 2H). LCMS (ESI^+) (*m*/*z*) 168 $[M+H]^+$.

A room temperature mixture of the product from the previous step (0.711 g, 4.25 mmol) in CH_2Cl_2 (30 mL) was treated with benzoyl isothiocyanate (833 mg, 5.10 mmol). The reaction was stirred at room temperature for 45 min under nitrogen. The solvent was removed to afford crude product that was purified on silica gel with a 0–100% EtOAc in hexanes gradient to afford (±)-**13** (1.273 g, 91% yield). R_f = 0.36 (1:1 hexanes/EtOAc, I_2 stain). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.90 (br s, 1H), 8.9 (br s, 1H), 7.81–7.78 (m, 2H), 7.61–7.59 (m, 1H), 7.50–7.48 (m, 2H), 5.97–5.93 (m, 1H), 5.45–5.34 (m, 2H), 4.73–4.70 (m, 1H), 2.53–2.51 (m, 1H), 2.34–2.33 (m, 3H), 1.94–1.93 (m, 1H), 1.76–1.75 (m, 2H). LCMS (ESI^+) (*m*/*z*) 331 $[M+H]^+$.

4.1.3. *N*-((4*RS*,4a*RS*,6*SR*,8a*SR*)-4-(Iodomethyl)-9-oxo-4,4a,5,6,7,8-hexahydro-6,8a-(epoxymethano)benzo[*d*][1,3]thiazin-2-yl)-benzamide ((±)-14)

A 0 °C solution of (±)-**13** (1.28 g, 3.87 mmol) in CH_2Cl_2 (70 mL) was treated with I_2 (1.97 g, 7.75 mmol) and the reaction was warmed to room temperature and stirred for 30 min under nitrogen. The red-brown reaction slurry was diluted with aqueous sodium bisulfite and extracted three times with EtOAc. The combined organic layers were dried (Na_2SO_4) and the solvent was removed to afford crude product that was purified with silica gel with a 5–100% EtOAc in hexanes gradient to afford the titled

product (1.323 g, 75% yield). R_f = 0.09 (1:1 hexanes/EtOAc). The relative stereochemistry was assigned based on the measured coupling constant of 11.1 Hz between the adjacent methane protons (H_4 and H_{4a}), which agreed with predicted coupling constants of the minimized structure of the isomer shown above. The predicted methine coupling constants is 11.0 Hz for the isomer shown and 3.5 Hz for the isomer with the opposite iodomethylene configuration. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–8.09 (m, 2H), 7.50–7.47 (m, 1H), 7.41–7.37 (m, 2H), 4.80–4.78 (m, 1H), 3.53–3.50 (m, 1H), 3.46–3.42 (m, 2H), 2.66–2.62 (m, 1H), 2.43–2.40 (m, 3H), 2.02–2.01 (m, 2H), 1.64–1.63 (m, 2H). LCMS (ESI$^+$) (*m*/*z*) 457 [M+H]$^+$.

4.1.4. *N*-((4*RS*,4a*RS*,6*SR*,8a*SR*)-6-Hydroxy-4,4a,5,6,7,8-hexahydro-1*H*,3*H*-4,8a-(epithiomethenoazeno)isochromen-10-yl)-benzamide ((±)-20)

A room temperature solution of (±)-**14** (1.341 g, 2.94 mmol) in 98:2 THF/water (97 mL) was treated quickly with a 2 M solution of $LiBH_4$ in THF (14.7 mL, 29.39 mmol), and the reaction was stirred at room temperature for 10 min under nitrogen. The reaction was quenched with MeOH (30 mL) and was stirred at room temperature for 2 min until a clear solution developed. The solvent was removed to afford crude product that was purified with silica gel with a step gradient of 75% EtOAc in hexanes to 100% EtOAc, then 10% MeOH in CH_2Cl_2 to afford the titled product (185 mg, 19% yield). R_f = 0.29 (9:1 CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.07–8.04 (m, 2H), 7.48–7.46 (m, 1H), 7.39–7.35 (m, 2H), 4.11–4.09 (m, 2H), 3.96–3.92 (m, 1H), 3.62–3.59 (m, 1H), 3.44–3.40 (m, 1H), 3.09–3.07 (m, 1H), 2.61–2.58 (m, 1H), 1.88–1.87 (m, 5H), 1.48–1.45 (m, 1H). ^{13}C (100 MHz, CD_3OD): δ 131.4, 128.8, 127.7, 76.6, 74.0, 64.4, 53.1, 42.8, 32.8, 26.6, 25.3. HRMS (ESI$^+$) (*m*/*z*) calcd for $C_{17}H_{21}N_2O_3S$ (M+H): 333.1273, found 333.1273.

4.1.5. *N*-((4*RS*,4a*RS*,8a*SR*)-6-Oxo-4,4a,5,6,7,8-hexahydro-1*H*,3*H*-4,8a-(epithiomethenoazeno)isochromen-10-yl)benzamide ((±)-21)

A room temperature solution of (±)-**20** (183 mg, 551 μmol) and *N*-methylmorpholine-*N*-oxide (129 mg, 1.10 mmol) in dry CH_2Cl_2 (16 mL) was treated with tetrapropylammonium perruthenate (19 mg, 550 μmol) and the reaction was stirred under nitrogen for 1 h. The solvent was removed, and the residue was purified on silica gel with a 0.5–10% MeOH in CH_2Cl_2 gradient to afford the titled product (154 mg, 85% yield). R_f = 0.37 (9:1 CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13–8.12 (m, 2H), 7.51–7.50 (m, 1H), 7.43–7.39 (m, 2H), 4.22–4.19 (m, 1H), 3.85–3.83 (m, 2H), 3.36–3.32 (m, 1H), 3.09–3.07 (m, 1H), 2.82–2.79 (m, 2H), 2.44–2.42 (m, 3H), 2.02–2.00 (m, 1H), 1.81–1.78 (m, 1H). ^{13}C (100 MHz, $CDCl_3$): δ 207.4, 132.2, 128.9, 128.3, 73.9, 68.1, 63.7, 51.9, 48.4, 42.6, 42.1, 37.1, 36.4, 33.9, 32.5, 22.7, 14.1. HRMS (ESI+) (*m*/*z*) calcd for $C_{17}H_{19}N_2O_3S$ (M+H): 331.1116, found 331.1111.

4.1.6. *N*-((4*RS*,4a*RS*,6*RS*,8a*SR*)-6-((*tert*-Butylsulfinyl)amino)-4,4a,5,6,7,8-hexahydro-1*H*,3*H*-4,8a-(epithiomethenoazeno)-isochromen-10-yl)benzamide ((±)-23)

A solution of (±)-**21** (114 mg, 345 μmol) and (±)-2-methylpropane-2-sulfinamide (150 mg, 414.03 μmol) in THF (6 mL) was treated with $Ti(OEt)_4$ (197 mg, 863 μmol) and then heated to 65 °C under nitrogen for 4 h. The reaction was cooled to −30 °C and treated portion-wise with $NaBH_4$ (25 mg, 690 μmol) and the reaction mixture was stirred for 5 min, then was quenched with MeOH (1 mL) and warmed to room temperature and stirred under nitrogen for 20 min. The reaction was treated with water, diluted with EtOAc, and the milky mixture was filtered through a small amount of diatomaceous earth using EtOAc to rinse. The filtrate was diluted with water and washed three times with EtOAc. The combined organic layers were dried (Na_2SO_4) and the solvent was removed to afford crude product that was purified on silica gel with a 15 min 0.5–10% MeOH in CH_2Cl_2 gradient to afford the title product (149 mg, 99% yield). R_f = 0.30 (9:1 CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20–8.18 (m, 2H), 7.51–7.47 (m, 1H), 7.42 (t, *J* = 7.4 Hz, 2H), 4.19–4.12 (m, 1H), 3.89–3.86 (m, 1H), 3.76 (d, *J* = 11.4 Hz, 2H), 3.39–3.30 (m, 1H), 3.22 (d, *J* = 5.7 Hz, 1H), 3.08 (d, *J* = 1.5 Hz, 1H), 2.11–2.03 (m, 3H), 1.77–1.67 (m, 4H), 1.20 (s, 9H). ^{13}C (100 MHz, $CDCl_3$): δ 131.9, 129.3, 128.1, 74.44, 74.40, 53.6, 53.0, 52.2, 52.1, 42.7, 42.6, 36.6, 36.3, 35.3, 34.1, 31.4, 31.2, 29.4, 28.6, 22.6, 22.1. HRMS (ESI$^+$) (*m*/*z*) calcd for $C_{21}H_{30}N_3O_3S_2$ (M+H): 436.1723, found 436.1725.

4.1.7. (+)-*N*-((4*S*,4a*S*,6*S*,8a*R*)-10-Amino-4,4a,5,6,7,8-hexahydro-1*H*,3*H*-4,8a-(epithiomethenoazeno)isochromen-6-yl)-3-chlorobenzamide (7)

A 0 °C solution of (±)-**23** (211 mg, 484 μmol) in CH_2Cl_2 (6 mL) was treated with a 1 N Et_2O solution of HCl (3.39 mL, 3.39 mmol). A white slurry developed that was warmed to room temperature and stirred under nitrogen for 30 min. The solvent was removed, and the crude product was split and purified in parallel on two 10 g SCX columns, each using 2:1 CH_2Cl_2 then 2:1 (CH_2Cl_2/7 N NH_3) in MeOH to elute the product. The solvent was removed from the basic washes to afford *N*-((4*RS*,4a*RS*,6*RS*,8a*SR*)-6-amino-4,4a,5,6,7,8-hexahydro-1*H*,3*H*-4,8a-(epithiomethenoazeno)isochromen-10-yl)benzamide (146 mg, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, *J* = 7.2 Hz, 2H), 7.50–7.47 (m, 1H), 7.42 (t, *J* = 7.3 Hz, 2H), 4.16 (d, *J* = 11.4 Hz, 1H), 3.85 (d, *J* = 11.3 Hz, 1H), 3.72 (d, *J* = 11.4 Hz, 1H), 3.28 (d, *J* = 11.4 Hz, 1H), 3.07 (s, 1H), 2.91–2.76 (m, 1H), 2.12–2.10 (m, 1H), 1.94–1.91 (m, 1H), 1.78 (d, *J* = 12.4 Hz, 1H), 1.73–1.63 (m, 1H), 1.58–1.41 (m, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 137.0, 131.7, 129.3, 128.0, 74.5, 52.2, 42.9, 36.7, 31.5. HRMS (ESI$^+$) (*m*/*z*) calcd for $C_{17}H_{22}N_3O_2S$ (M+H): 332.1433, found 332.1423.

A solution of the product from the previous step (146 mg, 441 μmol), 3-chlorobenzoic acid (83 mg; 529 μmol), and 1-hydroxybenzotriazole hydrate (88 mg; 573 μmol) in THF (4 mL) was treated with diisopropylethylamine (114 mg, 881 μmol). Then, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (110 mg, 573 μmol) was added, and the reaction was stirred at room temperature for 1.5 h under nitrogen. The reaction mixture was diluted with EtOAc, water, and saturated aqueous $NaHCO_3$, and then the combined aqueous layers were extracted three times with EtOAc. The combined organic layers were dried (Na_2SO_4) to give crude product that was purified on silica gel with a 0.5–10% MeOH in CH_2Cl_2 gradient to afford (−)-*N*-((4*S*,4a*S*,6*S*,8a*R*)-10-benzamido-4,4a,5,6,7,8-hexahydro-1*H*,3*H*-4,8a (epithiomethenoazeno)isochromen-6-yl)-3-chlorobenzamide (188 mg, 91% yield). R_f = 0.47 (9:1 CH_2Cl_2/MeOH). This material was subjected to chiral SFC (Column: Chiralpak AD-H, 2.1 cm × 15 cm; eluent: 40% isopropanol and 60% CO_2; flow 70 mL/min at UV 290 nm). Analysis of the first eluting isomer (Column: Chiralpak AD-H, 0.46 cm × 15 cm; 40% isopropanol and 60% CO_2; flow 5 mL/min at UV 225 nm) indicated that the product was isolated in >99% ee (t_R (major) = 1.3 min; t_R (minor) = 2.0 min), (69 mg, 43% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.6 (br s, 1H), 8.18–8.16 (m, 2H), 7.75 (t, *J* = 1.8 Hz, 1H), 7.60 (dt, *J* = 7.8, 1.2 Hz, 1H), 7.51–7.45 (m, 5H), 6.25 (d, *J* = 8.0 Hz, 1H), 4.20–4.13 (m, 2H), 3.89 (dd, *J* = 1.3, 11.7 Hz, 1H), 3.77 (d, *J* = 11.4 Hz, 1H), 3.35 (d, *J* = 11.5 Hz, 1H), 3.08–3.07 (m, 1H), 2.16 (dt, *J* = 12.3, 3.4 Hz, 1H), 2.06–1.97 (m, 2H), 1.79–1.67 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5, 136.0, 134.8, 131.9, 131.6, 129.9, 129.3, 128.1, 127.4, 124.9, 74.4, 52.1, 47.3, 42.7, 36.4, 32.7, 31.3, 27.0. HRMS (ESI$^+$) (*m*/*z*) calcd for $C_{24}H_{25}ClN_3O_3S$ (M+H): 470.1305, found 470.1302. $[\alpha]_D^{20}$ −181.70 (*c* 10, CH_2Cl_2).

A solution of the product from the previous step (69 mg, 147 μmol), *O*-methylhydroxylamine hydrochloride (123 mg, 1.5 mmol), and pyridine (116 mg; 1.5 mmol) in ethanol (4 mL) was heated to 50 °C overnight. The solvent was removed to afford a crude residue that was purified on silica gel with a 1–10% methanol (w/20% isopropylamine) in CH_2Cl_2 gradient to furnish **7** (50 mg, 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (t, J = 1.8 Hz, 1H), 7.63 (d, J = 7.7 Hz, 1H), 7.47 (ddd, J = 8.0, 2.0, 1.0 Hz, 1H), 7.37 (t, J = 7.9 Hz, 1H), 6.11 (d, J = 7.8 Hz, 1H), 4.15–4.07 (m, 2H), 3.81 (dd, J = 1.2, 11.5 Hz, 1H), 3.64 (d, J = 11.0 Hz, 1H), 3.22 (d, J = 10.9 Hz, 1H), 3.07 (d, J = 1.1 Hz, 1H), 1.93–1.86 (m, 3H), 1.68–1.58 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.4, 152.0, 136.4, 134.7, 131.5, 129.9, 127.3, 125.0, 74.7, 51.5, 48.1, 42.3, 34.0, 33.9, 33.2, 27.6, 21.1. HRMS (ESI$^+$) (m/z) calcd for $C_{17}H_{21}ClN_3O_2S$ (M+H): 366.1043, found 366.1042. $[\alpha]_D^{20}$ +3.70 (c 10, CH_2Cl_2).

4.1.8. (3a*RS*,7a*RS*)-7a-(((*tert*-Butyldimethylsilyl)oxy)methyl)-1,4,7,7a-tetrahydroisobenzofuran-3a(3*H*)-yl)methanol ((±)-25)

A 80 °C solution of lithium aluminum hydride (1.0 M in THF, 65.04 mL, 65.04 mmol) was treated dropwise with a solution of **24**[16] (8.1 g, 32.52 mmol) in THF (50 mL). The reaction stirred at 80 °C for 2 h and was then cooled to room temperature and quenched with $Na_2SO_4{\cdot}10H_2O$. Ethyl acetate and water were added, and the organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine and then dried over Na_2SO_4 and the solvent was removed to afford (*cis*-4,7-dihydroisobenzofuran-3a,7a(1*H*,3*H*)-diyl)dimethanol (5.49 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.68 (t, J = 1.8 Hz, 2H), 3.81 (s, 2H), 3.79 (d, J = 2.5 Hz, 2H), 3.65–3.63 (m, 2H), 3.58 (d, J = 11.5 Hz, 2H), 2.56–2.53 (m, 2H), 2.29–2.22 (m, 2H), 2.09–2.03 (m, 2H).

Sodium hydride (60% dispersion in mineral oil, 1.60 g, 39.89 mmol) was added to THF (50 mL). This suspension was treated dropwise with a solution of the product from the previous step (7.35 g, 39.9 mmol) in THF (50 mL). The reaction stirred at room temperature for 30 min and then *t*-butyldimethylchlorosilane (6.07 g, 39.89 mmol) was added to the reaction and it was stirred at room temperature for an additional 18 h. Ethyl acetate and water were added, and the organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine and then dried over Na_2SO_4 and the solvent was removed to afford crude product that was purified on silica gel with a 0–50% EtOAc in hexanes gradient to afford the titled product (9.87 g, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.70–5.67 (m, 2H), 3.81–3.72 (m, 4H), 3.62 (dd, J = 8.2, 5.3 Hz, 2H), 3.56 (d, J = 10.6 Hz, 1H), 3.51–3.47 (m, 1H), 3.33–3.29 (m, 1H), 2.32–2.27 (m, 2H), 2.09–2.03 (m, 2H), 0.89 (s, 9H), 0.07 (s, 6H).

4.1.9. (3a*RS*,7a*SR*)-7a-(((*tert*-Butyldimethylsilyl)oxy)methyl)-1,4,7,7a-tetrahydroisobenzofuran-3a(3*H*)-carboxylic acid ((±)-26)

A solution of (±)-**25** (9.87 g, 33.07 mmol) in CH_2Cl_2 (100 mL) was treated with pyridinium chlorochromate (14.55 g, 66.13 mmol) and the reaction was stirred at room temperature for two days. The reaction slurry was filtered through a pad of diatomaceous earth, and the filter cake was washed multiple times with CH_2Cl_2. The solvent was removed from the filtrate to afford crude product that was purified on silica gel with a 0–20% EtOAc in hexanes gradient to afford (3a*RS*,7a*SR*)-7a-(((*tert*-butyldimethylsilyl)oxy)methyl)-1,4,7,7a-tetrahydroisobenzofuran-3a(3*H*)-carbaldehyde (7.66 g, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.70 (s, 1H), 5.71 (t, J = 1.8 Hz, 2H), 4.17 (d, J = 8.9 Hz, 1H), 3.86 (d, J = 8.4 Hz, 1H), 3.68–3.61 (m, 3H), 3.46 (d, J = 10.3 Hz, 1H), 2.45–2.39 (m, 1H), 2.30–2.23 (m, 1H), 2.07–2.04 (m, 1H), 2.03–1.99 (m, 1H), 0.83 (s, 9H), −0.01 (s, 6H).

A 0 °C solution of the product from the previous step (7.66 g, 25.84 mmol) in THF (50 mL), *t*-butyl alcohol (75 mL) and 2-methyl-2-butene (50 mL) was treated with a freshly prepared solution of sodium chlorite (29.21 g, 258.36 mmol), and then a solution of monosodium phosphate monohydrate (35.65 g, 258.36 mmol) in water (100 mL) was added slowly to the solution. The solution was stirred at 0 °C for 30 min and was then warmed to room temperature and stirred for 16 h. The reaction was quenched with aqueous saturated ammonium chloride. Dichloromethane and water were added, and the organic layer was separated and the aqueous layer was extracted with more CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and the solvent was removed to afford crude product that was purified over silica gel with a 0–50% EtOAc in hexanes gradient to afford the titled product (8.58 g, 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73–5.72 (m, 2H), 4.27 (d, J = 8.6 Hz, 1H), 3.90 (d, J = 8.4 Hz, 1H), 3.70–3.55 (m, 4H), 2.53–2.51 (m, 1H), 2.34–2.29 (m, 1H), 2.03–1.98 (m, 1H), 1.27–1.18 (m, 1H), 0.85 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H). LCMS (ESI$^+$) (m/z) 313 [M+H]$^+$.

4.1.10. Benzyl ((3a*RS*,7a*RS*)-7a-(hydroxymethyl)-6-oxohexahydroisobenzofuran-3a(3*H*)-yl)carbamate ((±)-27)

(±)-**26** (7.61 g, 24.35 mmol), benzyl alcohol (3.95 g, 36.53 mmol), and diisopropylethylamine (12.59 g, 97.41 mmol) were dissolved in toluene (100 mL). Diphenylphosphonic azide (10.05 g, 36.53 mmol) was added, and the reaction was heated to 110 °C for 18 h. The reaction was cooled to room temperature. Ethyl acetate and water were added. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, and the solvent was removed to afford crude product that was purified on silica gel with a 0–30% EtOAc in hexanes gradient to afford benzyl ((3a*RS*,7a*SR*)-7a-(((*tert*-butyldimethylsilyl)oxy)methyl)-1,4,7,7a-tetrahydroisobenzofuran-3a(3*H*)-yl)carbamate (7.82 g, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32–7.22 (m, 5H), 5.60 (s, 2H), 5.02 (s, 2H), 4.28–4.26 (m, 1H), 4.06–4.02 (m, 1H), 3.85–3.81 (m, 2H), 3.66–3.59 (m, 2H), 2.75–2.58 (m, 2H), 2.19–2.02 (m, 2H), 0.84 (s, 9H), 0.057 (s, 3H), 0.046 (s, 3H). LCMS (ESI$^+$) (m/z) 418 [M+H]$^+$.

A 0 °C solution of the product from the previous step (3.36 g, 8.05 mmol]) in THF (50 mL) was treated with borane–methyl sulfide complex (3.06 g, 40.23 mmol) and the reaction was stirred at 0 °C for 1 h and was then warmed to room temperature and stirred for 2 h. Aqueous 1 N NaOH (50 mL) was added slowly to the reaction and the reaction was stirred for 30 min. Hydrogen peroxide (30% aqueous solution, 30 mL, 295.81 mmol) was then added, and the reaction was stirred at room temperature for 2 h. The reaction was diluted with (3:1 CH_2Cl_2/isopropanol) and water. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and the solvent was removed to afford crude product that was purified on silica gel with a 0–50% EtOAc in hexanes gradient to afford 2.52 g (72%) of an intermediate regioisomeric mixture of alcohols that was dissolved in CH_2Cl_2 (20 mL) and treated with 3,3,3-triacetoxy-3-iodophthalide (5.06 g, 11.57 mmol) and the reaction was stirred at room temperature for 4 h. Dichloromethane and water were added and the two-phased mixture was filtered through a pad of diatomaceous earth to remove insoluble materials. From the filtrate, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, and the solvent was removed to afford crude product that was purified on silica gel with a 0–50% EtOAc in hexanes gradient to afford a 1:1 mixture of regioisomeric ketones that was carried on as is (2.20 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31–7.25 (m, 5H), 5.04–4.98 (m, 2H), 4.39–4.36 (m, 0.5H), 4.23–4.22 (m, 0.5H), 3.99–3.83 (m, 2H), 3.77 (d, J = 9.5 Hz, 0.5H), 3.60 (d, J = 9.4 Hz, 0.5H), 3.54–3.49

(m, 1H), 3.38–3.35 (m, 0.5H), 3.29 (d, J = 10.6 Hz, 0.5H), 3.02–3.01 (m, 0.5H), 2.80–2.76 (m, 0.5H), 2.56–2.50 (m, 4H), 2.10–2.01 (m, 0.5H), 1.85–1.83 (m, 0.5H), 0.83 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). LCMS (ESI$^+$) (m/z) 434 [M+H]$^+$.

The product from the previous step (2.198 g, 5.07 mmol) was dissolved in THF (10 mL) and treated with a 1 M solution of tetrabutylammonium fluoride in THF (10.14 mL, 10.14 mmol), and the reaction was stirred under nitrogen for 4 h. Dichloromethane and water were added, and the organic layer was separated, and the aqueous layer was extracted with more CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, and the solvent was removed to afford crude product that was purified on silica gel with a 25–100% EtOAc in hexanes gradient to afford (±)-**27** as a single isomer (813 mg, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.34 (m, 5H), 6.85–6.81 (m, 1H), 5.06 (s, 2H), 4.44–4.43 (m, 1H), 3.99 (dd, J = 3.1, 10.5 Hz, 2H), 3.63 (d, J = 9.3 Hz, 1H), 3.47–3.40 (m, 2H), 2.62 (d, J = 15.3 Hz, 1H), 2.57–2.55 (m, 5H), 2.13–2.08 (m, 1H). LCMS (ESI$^+$) (m/z) 320 [M+H]$^+$.

4.1.11. *N*-(((3a*RS*,7a*RS*)-7a-(Hydroxymethyl)-6-oxohexahydroisobenzofuran-3a(3*H*)-yl)carbamothioyl)benzamide ((±)-28)

A Parr bottle was charged with (±)-**27** (1.064 g, 3.33 mmol), 10% Pd/C (0.5520 g), EtOAc (80 mL), and EtOH (80 mL) and was purged with nitrogen (4×), purged with hydrogen (4×) and then pressurized with hydrogen (60 psi g) and was shaken at room temperature for 10 min. The mixture was vented, and the catalyst was removed by filtration, using EtOH to rinse. The filtrate was treated with benzoyl isothiocyanate (1.09 g, 6.66 mmol), and the reaction was stirred at room temperature for 2 h. The solvent was removed, and the crude product was purified over silica gel with a 0–50% EtOAc in hexanes gradient to afford the titled product (885 mg, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.49–11.45 (m, 1H), 8.92–8.91 (m, 1H), 7.82–7.80 (m, 2H), 7.65–7.61 (m, 1H), 7.53–7.50 (m, 2H), 4.79 (d, J = 10.1 Hz, 1H), 4.28 (d, J = 10.1 Hz, 1H), 4.14–4.07 (m, 1H), 3.68–3.61 (m, 2H), 3.48 (d, J = 9.3 Hz, 1H), 3.24–3.18 (m, 1H), 2.70 (d, J = 15.6 Hz, 1H), 2.56 (d, J = 15.6 Hz, 1H), 2.49–2.41 (m, 4H). LCMS (ESI$^+$) (m/z) 349 [M+H]$^+$.

4.1.12. *N*-((4a*RS*,8a*SR*)-6-Oxo-5,6,7,8-tetrahydro-4*H*-4a,8a-(methanooxymethano)benzo[*d*][1,3]thiazin-2-yl)benzamide ((±)-29)

A 0 °C solution of (±)-**28** (880 mg, 2.53 mmol) in CH_2Cl_2 (10 mL) was treated with 1-chloro-*N*,*N*,2-trimethylpropenylamine (506 mg, 3.79 mmol) and then the reaction was warmed to room temperature and stirred under nitrogen for 18 h. Dichloromethane and water were added, and the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts dried over Na_2SO_4, and the solvent was removed to afford crude product that was purified over silica gel with a 0–100% EtOAc in hexanes gradient to afford the titled product (758 mg, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12–8.06 (m, 2H), 7.51 (t, J = 7.3 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 4.03–3.97 (m, 2H), 3.90 (t, J = 9.0 Hz, 2H), 3.15 (d, J = 13.6 Hz, 1H), 2.91–2.87 (m, 1H), 2.83–2.77 (m, 2H), 2.70–2.66 (m, 1H), 2.56–2.50 (m, 2H), 2.33–2.22 (m, 2H). LCMS (ESI$^+$) (m/z) 331 [M+H]$^+$.

4.1.13. *N*-((4a*RS*,8a*SR*)-6-Amino-5,6,7,8-tetrahydro-4*H*-4a,8a-(methanooxymethano)benzo[*d*][1,3]thiazin-2-yl)benzamide ((±)-30)

A solution of (±)-**29** (500 mg, 1.51 mmol) was dissolved in THF (5 mL) and then treated sequentially with (±)-2-methyl-2-propanesulfinamide (220 mg, 1.82 mmol) and Ti(OEt)$_4$ (863 mg, 3.78 mmol), and the reaction was heated to 65 °C for 5 h. The reaction was cooled to room temperature and then to 0 °C. Sodium borohydride (115 mg, 3.03 mmol) was added, followed by MeOH (4 mL). The reaction was warmed to room temperature and stirred for 1 h. Ethyl acetate and water were added. The solution was then filtered through diatomaceous earth, and the filter cake was washed with EtOAc and water. The filtrate was collected, and the organic layer was separated and aqueous layer was extracted with EtOAc (3×). The organic extracts were combined, dried over Na_2SO_4, filtered, and the solvent was removed to afford crude product that was purified on silica gel with a 25–100% EtOAc in hexanes gradient to afford *N*-((4a*RS*,8a*SR*)-6-((1,1-dimethylethyl) sulfonamido)-5,6,7,8-tetrahydro-4*H*-4a,8a-(methanooxymethano) benzo[*d*][1,3]thiazin-2-yl)benzamide as a ca. 3:2 mixture of C(6) epimers (346 mg, 52% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14–8.08 (m, 2H), 7.51–7.50 (m, 1H), 7.43–7.38 (m, 2H), 4.24–4.21 (m, 1H), 4.18–4.14 (m, 0.4H), 3.91–3.84 (m, 2H), 3.75–3.72 (m, 0.6H), 3.57–3.55 (m, 1.6H), 3.29–3.24 (m, 0.4H), 3.11–3.07 (m, 1H), 2.87–2.83 (m, 0.4H), 2.51 (dd, J = 13.6, 3.6 Hz, 0.6H), 2.05–2.00 (m, 6H), 1.22–1.21 (m, 3H), 1.19 (s, 3H), 1.18 (s, 3H). LCMS (ESI$^+$) (m/z) 436 [M+H]$^+$.

A solution of the product from the previous step (346 mg, 794 μmol) in diethyl ether (5 mL) and CH_2Cl_2 (~1 mL) was treated with a 1 N Et_2O solution of HCl (4 mL, 4 mmol). The reaction was stirred at room temperature for 3 h. The solvent was removed, and the crude product was purified sequentially with a SCX column using MeOH then (7 N NH_3 in MeOH) to flush the product off the SCX column. The basic extract was concentrated, and the product was purified again on silica with a 0–10% (7 N NH_3 MeOH) in CH_2Cl_2 gradient to furnish (±)-**30** as a 1:1 mixture of C(6) epimers (215 mg, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14–8.07 (m, 2H), 7.49–7.44 (m, 1H), 7.41–7.36 (m, 2H), 4.29–4.25 (m, 0.5H), 4.18 (dd, J = 9.2, 6.5 Hz, 1H), 3.90–3.79 (m, 2H), 3.69 (d, J = 9.1 Hz, 0.5H), 3.53 (d, J = 13.5 Hz, 0.5H), 3.31 (d, J = 13.6 Hz, 0.5H), 3.06–2.99 (m, 0.5H), 2.96–2.89 (m, 0.5H), 2.77–2.72 (m, 0.5H), 2.49 (d, J = 13.6 Hz, 0.5H), 2.17–2.09 (m, 0.5H), 1.99–1.92 (m, 4.5H), 1.41–1.20 (m, 2H), 0.88–0.79 (m, 2H). LCMS (ESI$^+$) (m/z) 332 [M+H]$^+$.

4.1.14. *N*-((4a*RS*,6*RS*,8a*SR*)-2-Amino-5,6,7,8-tetrahydro-4*H*-4a,8a-(methanooxymethano)benzo[*d*][1,3]thiazin-6-yl)-3-chlorobenzamide ((±)-8)

A 0 °C mixture of (±)-**30** (107 mg, 323 μmol), 3-chlorobenzoic acid (104 mg, 646 μmol), 1-hydroxybenzotriazole (65 mg, 384 μmol), and *N*,*N*-diisopropylethylamine (83 mg, 113 μL, 646 μmol) in THF (5 mL) was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (124 mg, 646 μmol), and the reaction was warmed to room temperature and stirred for 18 h. The reaction was diluted with water and extracted multiple times with CH_2Cl_2. The combined organics layers were dried ($MgSO_4$), filtered, and the solvent was removed to afford crude product that was purified on silica gel with a 0–100% EtOAc in hexanes gradient to afford *N*-((4a*RS*,6*RS*,8a*RS*)-2-benzamido-5,6,7,8-tetrahydro-4*H*-4a,8a-(methanooxymethano)benzo[*d*][1,3]thiazin-6-yl)-3-chlorobenzamide (37 mg, 24% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, J = 7.2 Hz, 2H), 7.70 (t, J = 1.9 Hz, 1H), 7.57 (ddd, J = 7.7, 1.6, 1.2 Hz, 1H), 7.49–7.45 (m, 5H), 6.09–6.04 (m, 1H), 4.34 (d, J = 9.3 Hz, 1H), 4.31–4.30 (m, 1H), 3.91–3.84 (m, 2H), 3.75 (d, J = 9.3 Hz, 1H), 3.56 (d, J = 13.7 Hz, 1H), 2.48 (d, J = 13.6 Hz, 1H), 2.13–2.11 (m, 1H), 2.02–1.97 (m, 4H), 1.55–1.54 (m, 1H). LCMS (ESI$^+$) (m/z) (^{35}Cl/^{37}Cl) 470/472 [M+H]$^+$.

A mixture of the product from the previous step (37 mg, 78.7 μmol), pyridine (63 mg, 64 μL, 787 μmol), and *O*-methylhydroxylamine hydrochloride (67 mg, 787 μmol) in ethanol (3 mL) was heated to 50 °C for 17 h. The reaction was cooled to room temperature, and the solvent was removed to afford crude product that was purified over silica gel with a 0–10% (7 N NH_3 methanol) in CH_2Cl_2 gradient to afford (±)-**8** (27 mg, 94% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (d, J = 7.9 Hz, 1H), 7.91 (t, J = 1.9 Hz, 1H), 7.81 (dt, J = 7.8, 1.3 Hz, 1H), 7.57 (ddd, J = 8.0, 2.1, 1.1 Hz, 1H), 7.47 (t, J = 7.8 Hz, 1H), 5.89–5.85 (br s, 2H), 4.15 (d,

J = 8.8 Hz), 4.01 (m, 1H), 3.59 (d, J = 8.8 Hz, 1H), 3.57 (d, J = 7.7 Hz, 1H), 3.45 (d, J = 7.7 Hz, 1H), 3.26 (d, J = 13.2 Hz, 1H), 2.54 (d, J = 13.2 Hz, 1H), 2.00 (t, J = 12.9 Hz, 1H), 1.81 (dt, J = 13.5, 2.5 Hz, 1H), 1.74 (dt, J = 13.5, 3.3 Hz, 1H), 1.60 (m, 2H), 1.31 (dq, J = 12.4, 3.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8, 147.2, 136.5, 133.0, 130.9, 130.2, 127.0, 126.1, 78.9, 75.4, 60.5, 44.7, 35.3, 34.1, 33.2, 31.4, 27.7. HRMS (ESI$^+$) (m/z) calcd for $C_{17}H_{20}ClN_3O_2S$ (M+H): 365.0965, found 365.0968.

4.2. Bioanalytical methods

Aliquots of homogenized brain or plasma samples and appropriate calibration standards were mixed with methanol/acetonitrile (1:1, v/v) containing internal standard to precipitate proteins, then centrifuged to pellet the precipitant. A volume of each sample's supernatant was transferred to a new 96-well plate and diluted 4-fold with water/methanol (1:1, v/v) then analyzed by LC–MS/MS using a Betasil C_{18} (Thermo) 20 mm × 2.1 mm × 5 μm Javelin column and a gradient mobile phase system with A: water/trifluoroacetic acid/1 M ammonium bicarbonate (1000:4:1, v/v) and B: acetonitrile/trifluoroacetic acid/1 M ammonium bicarbonate (1000:4:1, v/v) delivered at 1.5 mL/min. Selected Reaction Monitoring (SRM) transitions (positive ion mode) with for **6** (m/z 324.1 > 139.1), **7** (m/z 366.1 > 139.1), and internal standard (m/z 249.1 > 148.1) were acquired with an Applied Biosystems/MDS Sciex API4000 tuned to achieve unit resolution (0.7 Da at 50% FWHM) using Analyst software (version 1.4.2).

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Supplementary data

Supplementary data (experimental procedures for **4**·HCl, **5**·HCl, **6**·HCl, **10/12**, **16**, and **31**·TFA, analytical data, and in vitro selectivity and physicochemical data for compounds **4**, **5–8**, and **31**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.04.062.

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11. Full conformational analysis performed with MacroModel using the OPLS2.1 force field in the gas phase. All conformers within 10 kcal/mol of the minima were examined. Only the bound conformation was found for **7**, and an alternate ring conformation for **8** was 2.2 kcal/mol above the minima *Schrödinger Release 2013-2: MacroModel, version 10.1*; Schrödinger, LLC: New York, NY, 2013.
12. LEAN = $(-\log IC_{50})/N$, where N is the number of heavy (non-hydrogen) atoms and IC_{50} is in M.
13. **9** was prepared as a single isomer. The absolute stereochemistry is unknown; relative stereochemistry is as depicted.
14. See Supporting information for details.
15. See Supporting information for stereochemical analysis.
16. To estimate the transition state energy differences for the two cyclization pathways, a reaction coordinate scan was carried out using the program Jaguar (*Jaguar, version 8.3*, Schrödinger, LLC: New York, NY, 2014). Beginning with the UDFT (m06-2×)/3-21G* geometry optimized iodonium ion intermediates, a relaxed coordinate scan of the S–C distance from 1.8 to 3.6 Å was carried out. The UDFT (m06-2×) hybrid functional developed by Truhlar (Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.* **2008**, *120*, 215) was shown previously to work well with iodonium ions (George, L.; Kalume, A.; Reid, S. A. *Chem. Phys. Lett.* **2012**, *554*, 86). Maxima were found at 3.0 Å for the favored attack; that is, **15a** and 3.2 Å for **15b**. The energy difference for these two maxima was 5.7 kcal/mol in favor of the cyclization leading to the desired isomer, **14**.
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