Supporting Information

Structure-Based Design of an Iminoheterocyclic β-Site Amyloid Precursor Protein Cleaving Enzyme (BACE) Inhibitor that Lowers Central A*β* in non-Human Primates

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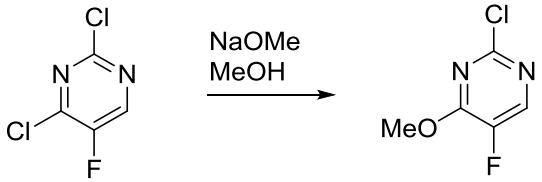
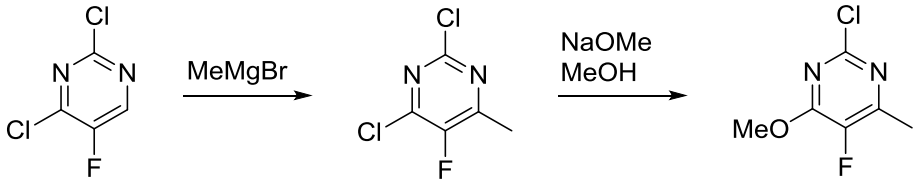
Synthesis of 2-chloro-5-fluoro-4-methoxypyrimidine.



To a solution of 2,4-dichloro-5-fluoropyrimidine (5.6 g, 34 mmol) in a mixture of THF and methanol (75 mL each) was added 25% sodium methoxide in methanol (7.3 g, 34 mmol) at 0 °C, and the resulting solution was stirred for 0.5 h. The reaction mixture was diluted with brine, extracted with ethyl acetate, and the organic layer was dried with $MgSO_4$, filtered and concentrated. The crude product was purified via silica gel chromatography using 0% to 50% CH_2Cl_2 in hexanes to provide 2-chloro-5-fluoro-4-methoxypyrimidine. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 2.2 Hz, 1H), 4.10 (s, 3H).

Synthesis of 2-chloro-5-fluoro-4-methoxy-6-methylpyrimidine.

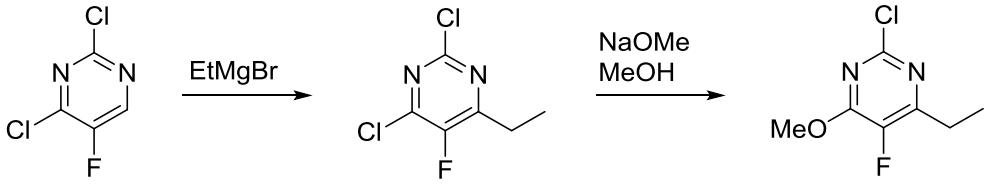


To a solution of MeMgBr (15 mL, 3.0 M in ether, 45 mmol) in THF (20 mL) was added a solution of 2,4-dichloro-5-fluoropyrimidine (5 g, 30 mmol) in DME (20 mL) while maintaining the temperature below 15 ^{o}C. The resulting solution was stirred at 15 ^{o}C for 1 h and then was cooled to 0 ^{o}C. A solution of triethyl amine (4.17 mL, 30 mmol) in 10 mL THF was added slowly to the reaction mixture maintaining the internal temperature ~ 5 ^{o}C, then was added a solution of iodine (7.5 g, 30 mmol) in 10 mL THF. The reaction mixture was quenched with water, warmed to room temperature, extracted with ethyl acetate, and the organic layer was

concentrated. The crude product was purified via silica gel chromatography using 0% to 100% dichloromethane / hexanes to afford 2,4-dichloro-5-fluoro-6-methylpyrimidine. ^{1}H NMR ($CDCl_3$) δ 2.42 (3H, d, J = 2.8 Hz).

To a solution of 2,4-dichloro-5-fluoro-6-methylpyrimidine (0.56 g, 3.11 mmol) in THF (6 mL) was added 25% sodium methoxide in methanol (0.671 g, 3.11 mmol) while cooling at 0 ^{o}C. The resulting solution was slowly warmed to room temperature over 1 h and then diluted with water and extracted with ethyl acetate The organic layer was concentrated, and purification of the crude material via silica gel chromatography using 0% to 100% ethyl acetate in hexanes provided the 2-chloro-5-fluoro-4-methoxy-6-methylpyrimidine (268 mg, 49% for two steps from 2,4-dichloro-5-fluoropyrimidine). ^{1}H NMR ($CDCl_3$) δ 3.1 (s, 3H), 2.42 (d, 3H, J = 2.8 Hz).

Synthesis of 2-chloro-4-ethyl-5-fluoro-6-methoxypyrimidine.

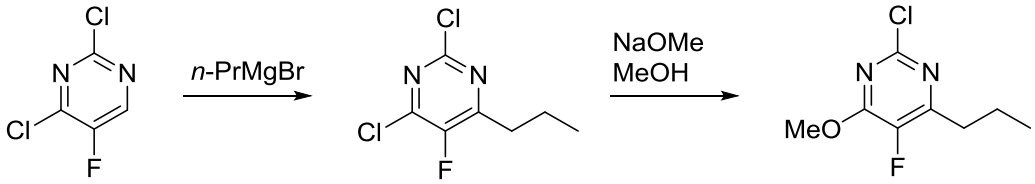


To a solution of EtMgBr (225 mL, 1.0 M in THF) was added a solution of 2,4-dichloro-5-fluoropyrimidine (25 g, 150 mmol) in DME (75 mL) while maintaining the temperature below 15 ^{o}C. The resulting solution was stirred at 15 ^{o}C for 1 h and then was cooled to 0 ^{o}C. A solution of triethyl amine (17 g, 168 mmol) in THF (35 mL) was added slowly to the reaction mixture maintaining the internal temperature ~ 5 ^{o}C, then a solution of iodine (38 g, 150 mmol) in THF (128 mL) was added. The reaction mixture was quenched with water, warmed to room temperature, extracted with ethyl acetate, and the organic layer was concentrated. The crude product was purified via silica gel chromatography using 0% to 100% dichloromethane / hexanes

mixtures to afford 2,4-dichloro-6-ethyl-5-fluoropyrimidine (2.4 g, 10%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 (qd, *J* = 7.6, 2.2 Hz, 2H), 1.31 (t, *J* = 7.6 Hz, 3H).

To a solution of 2,4-dichloro-6-ethyl-5-fluoropyrimidine (500 mg, 2.59 mmol) in THF (5 mL) was added 25% sodium methoxide in methanol (671 mg, 3.10 mmol) at 0 °C. The resulting solution was slowly warmed to room temperature over 1 h and then diluted with water and extracted with ethyl acetate. The organic layer was concentrated and subjected to silica gel chromatography using 0% to 100% ethyl acetate in hexanes to afford 2-chloro-4-ethyl-5-fluoro-6-methoxypyrimidine. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.06 (s, 3H), 2.76 (qd, *J* = 7.6, 2.6 Hz, 2H), 1.26 (t, *J* = 7.6, 3H).

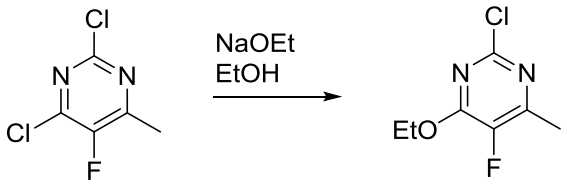
Synthesis of 2-chloro-5-fluoro-4-methoxy-6-propylpyrimidine.



To a solution of *n*-PrMgBr (23 mL, 2.0 M in THF, 46 mmol) was added a solution of 2,4-dichloro-5-fluoropyrimidine (5 g, 30 mmol) in DME (15 mL) while maintaining the temperature below 15 ^{o}C. The resulting solution was stirred at 15 ^{o}C for 1 h and then was cooled to 0 ^{o}C. A solution of triethylamine (4.3 mL, 30 mmol) in THF (8 mL) was added slowly to the reaction mixture maintaining the internal temperature ~ 5 ^{o}C followed by a solution of iodine (7.8 g, 30.8 mmol) in THF (20 mL). The reaction mixture was quenched with water, warmed to room temperature, extracted with ethyl acetate, and the organic layer was concentrated. The crude product was purified via silica gel chromatography using 0% to 100% dichloromethane / hexanes to afford 2,4-dichloro-5-fluoro-6-propylpyrimidine (500 mg, 10%).

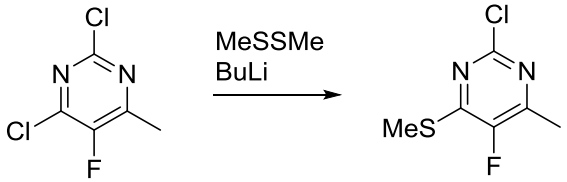
To a solution of 2,4-dichloro-5-fluoro-6-propylpyrimidine (0.500 g, 2.43 mmol) in THF (6 mL) was added 25% sodium methoxide in methanol (0.523 g, 2.43 mmol) while cooling at 0 ^{o}C. The resulting solution was slowly warmed to room temperature over 1 h and then diluted with water and extracted with ethyl acetate. The organic layer was concentrated and subjected to silica gel purification using 0% to 100% ethyl acetate in hexanes to provide 2-chloro-5-fluoro-4-methoxy-6-propylpyrimidine. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.05 (s, 3H), 2.75 – 2.64 (m, 2H), 1.79 – 1.64 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H).

Synthesis of 2-chloro-4-ethoxy-5-fluoro-6-methylpyrimidine.



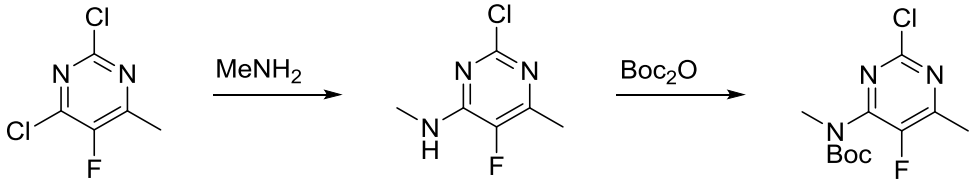
To a solution of 2,4-dichloro-5-fluoro-6-methylpyrimidine (1 g, 5.5 mmol) in THF (6 mL) was added 21% sodium ethoxide in ethanol (2.49 mL, 6.70 mmol) while cooling at 0 ^{o}C. The resulting solution was slowly warmed to room temperature over 1 h and then diluted with water and extracted with ethyl acetate. The organic layer was concentrated and subjected to silica gel purification using 0 to 100% ethyl acetate in hexanes to afford 2-chloro-4-ethoxy-5-fluoro-6-methylpyrimidine (250 mg, 23%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49 (q, *J* = 7.1, 2H), 2.42 (d, *J* = 3.0, 3H), 1.43 (t, *J* = 7.1 Hz, 3H).

Synthesis of 2-chloro-5-fluoro-4-methyl-6-(methylthio)pyrimidine.



To a solution of dimethyl disulfide (0.21 mL, 2.4 mmol) in diethyl ether (14 mL) at -78 °C was added n-BuLi in hexanes (2.5 M, 0.95 mL, 2.4 mmol). The resulting mixture was stirred for 0.5 h, and then a solution of 2,4-dichloro-5-fluoro-6-methylpyrimidine (0.4 g, 2.2 mmol) in diethyl ether (1 mL) was added. The resulting mixture was gradually warmed to 5 °C over 3 h and then diluted with water. The organic layer was separated, and the aqueous layer was extracted with ether (3x). The organic layers were combined, dried with $MgSO_4$, and concentrated. The resulting solid was triturated with hexanes to afford 2-chloro-5-fluoro-4-methyl-6-(methylthio)pyrimidine (267 mg, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.58 (s, 3H), 2.43 (d, J = 2.8 Hz, 3H).

Synthesis of *tert*-butyl (2-chloro-5-fluoro-6-methylpyrimidin-4-yl)(methyl)carbamate.

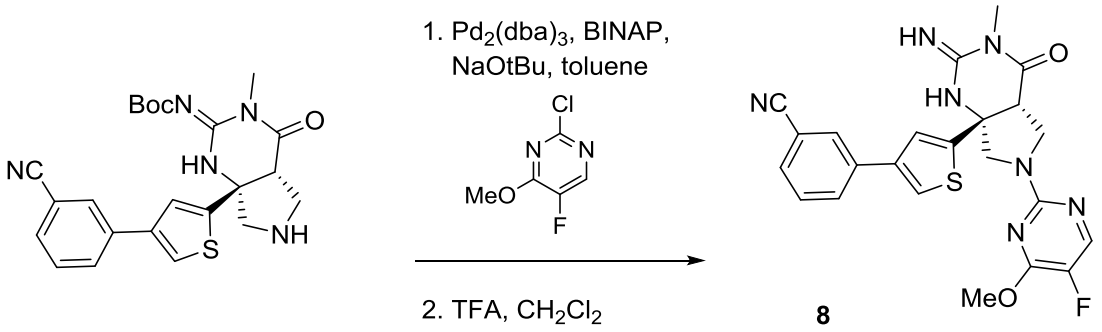


To a solution of 2,4-dichloro-5-fluoro-6-methylpyrimidine (0.5 g, 2.8 mmol) in THF (1.5 mL) at -20 °C was added DIEA (1 mL, 5.6 mmol) followed by methyl amine (2 M solution in THF, 1.6 mL, 3.3 mmol). The reaction mixture was slowly warmed to rt over 12 hr and was then diluted with water and extracted with ethyl acetate. The organic layer was dried with $MgSO_4$ and concentrated. The crude material was purified by flash chromatography using 20% ethyl

acetate in hexanes to provide 2-chloro-5-fluoro-N,6-dimethylpyrimidin-4-amine in quantitative yield.

To a solution of 2-chloro-5-fluoro-N,6-dimethylpyrimidin-4-amine (110 mg, 0.628 mmol) in CH_2Cl_2 (3 mL) was added $(Boc)_2O$ (410 mg, 1.8 mmol) and DMAP (8 mg, 0.07 mmol). The reaction mixture was stirred at rt for 12 h, and was then directly loaded onto a silica gel column and purified using 20% ethyl acetate in hexanes (43 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.32 (s, 3H), 2.48 (d, J = 3.0 Hz, 3H), 1.46 (s, 9H).

Synthesis of 3-(5-((4a*R*,7a*R*)-6-(4-fluoro-5-methoxypyrimidin-2-yl)-2-imino-3-methyl-4-oxooctahydro-7aH-pyrrolo[3,4-*d*]pyrimidin-7a-yl)thiophen-3-yl)benzonitrile (8).



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To a solution of *tert*-butyl ((4a*R*,7aR,E)-7a-(4-(3-cyanophenyl)thiophen-2-yl)-3-methyl-4-oxooctahydro-2H-pyrrolo[3,4-d]pyrimidin-2-ylidene)carbamate (0.35 g, 0.77 mmol), prepared as previously described,[1] and 2-chloro-5-fluoro-4-methoxypyrimidine (0.16 g, 1.0 mmol) in toluene (3 mL) was added tris(dibenzylideneacetone)dipalladium(0) (0.10 g, 0.15 mmol), BINAP (0.072 g, 0.15 mmol) and NaO*t*Bu (0.223 g, 2.32 mmol). The reaction mixture was degassed and heated under N_2 at 70 °C for 3 h, then cooled to room temperature, filtered through a pad of Celite, and the filtrate was concentrated. The crude material was purified by silica gel chromatography using 0% to 100% EtOAc/Hexanes to give a product which was directly treated with 20% TFA/CH_2Cl_2 (2 mL). The deprotected compound was purified by reverse phase C18

column using 0 to 90% acetonitrile (0.05% TFA) in water (0.05% TFA) to provide 3-(5-((4a*R*,7a*R*)-6-(5-fluoro-4-methoxypyrimidin-2-yl)-2-imino-3-methyl-4-oxooctahydro-7aH-pyrrolo[3,4-*d*]pyrimidin-7a-yl)thiophen-3-yl)benzonitrile **8** (59 mg, 16%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.07 - 8.02 (m, 2H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.88 (d, *J* = 1.5 Hz, 1H), 7.69 - 7.64 (m, 2H), 7.59 (t, *J* = 8.1 Hz, 1H), 4.46 (d, *J* = 12.1 Hz, 1H), 4.21 (dd, *J* = 8.0, 10.5 Hz, 1H), 4.16 - 4.10 (m, 2H), 4.07 - 4.01 (m, 1H), 3.34 (s, 4H). *m/z*: 478.3.

CYP 3A4 Inhibition Assay

In order to assess the potential for inhibition of CYP 3A4, human liver microsomes (0.4 mg/ml) were incubated with several concentrations of test article (0 to 50 μM), 1 mM NADPH, and substrates for various CYPs at 37°C for 10-20 minutes, depending on the enzyme reaction, in a buffer composed of 50 mM Tris-acetate, pH 7.4, and 150 mM potassium chloride. The test article was dissolved in methanol at a concentration of 5 mM. Dilutions of the stock solution were also prepared in methanol. The substrate concentration was kept near the Km value for each CYP reaction. The CYP3A4 substrates were 100 μM testosterone (6β-hydroxylase reaction) and 5 μM midazolam (1′-hydroxylase reaction). The reactions were terminated by the addition of 35% perchloric acid to a final concentration of 4.5% (vol:vol). The concentrations of the metabolites formed from each substrate after incubation were determined by LC-MS/MS using a standard curve. The concentration of test article that inhibits 50% of the initial enzyme activity (IC_{50}) values was determined from the graph of test article concentrations versus percent of inhibition.

To evaluate time-dependent metabolism/mechanism-based inhibition, the test article, at the stated concentrations (0 to 50 μM), was pre-incubated with human liver microsomes for 30 min at 37°C in the presence of NADPH and in the absence of the substrates. After the pre-incubation step, the CYP substrates were added at the previously stated concentrations and the reactions were allowed to proceed as indicated in the previous paragraph.

Incubation of compound 6 with CYP3A4 in the presence and absence of glutathione

Incubation: Compound **6** was incubated at 20 µM with recombinant CYP3A4 at 250 pmol/mL in 100 mM potassium phosphate buffer with 2 mM $MgCl_2$ (pH 7.4). Incubations were performed with and without reduced glutathione as trapping agent. Where used, glutathione was added to give a final concentration of 5 mM. The incubations were performed in a shaking water bath maintained at 37 °C. NADPH was added (final concentration 1 mM) to initiate the reactions. Samples were taken at 0 and 60 minutes for analysis. The reactions were quenched using equal volumes of cold acetonitrile.

LC-HRMS analysis: Incubated samples were analyzed using a Waters Xevo G2 Q-ToF high resolution mass spectrometer interfaced to a Waters Acquity UPLC system (Waters, Milford, MA). Experiments were conducted using full scan mass spectrometry (50 – 1000 Da) in centroid mode. Data was collected using both low (3V) and high (15-40 V ramp) collision energies. Samples were analyzed using electrospray ionization (ESI) interface scanning in the positive ion mode, with 0.2 second scans, operating at a source temperature of 120 °C and a desolvation temperature of 450 °C. Nitrogen and argon were used for the cone and collision cell gases, respectively. Leucine enkephalin was used as lock mass. Metabolites were assigned based on fragmentation patterns elucidated from the observed mass spectra.

Analyte separation was achieved through gradient elution using a Phenomenex Kinetics PFP 2.6 µM, 2.1 x 100 mm analytical column (Phenomenex, Torrance, CA) maintained at 40 °C. Mobile phases were A: 95:4:1, H_2O:acetonitrile: methanol (v:v:v), 10 mM ammonium acetate, 60 µL/L acetic acid; and B: 76:19:5 acetonitrile: methanol: H_2O (v:v:v) 10 mM ammonium acetate, 60 µL/L acetic acid. The gradient started at 0% B for 2 minutes, followed by a series of linear increases from 0% B to 5% B in 1 min, 5% B to 50% B in 9 min, 50% B to 95% B in 099

min. The gradient was held at 95% B for 0.81 min and then returned to the initial condition of 0% B in 0.1 min, which was held for 1.1 min.

Determination of monkey CSF and cortex Aβ_{40}, Aβ_{42} and sAPPβ levels

a. Cynomolgus monkey CSF and cortex sample preparation

Cynomolgus monkeys (n = 4) were sedated with ketamine and Telazol and CSF was collected from the cisterna magna by using a hand-held 22-gauge spinal needle. Four hours after oral dosing of compound **9** (formate salt; solution in 0.4% hydroxypropylmethylcellulose), the animals were given a lethal dose of anesthetic, CSF and blood were collected, whole brains were removed and a 1 cm thick section was cut from the outer-right and outer-left hemispheres. Brain sections were frozen on dry ice and stored at -80 ºC under vacuum until analysis. The control CSF samples were CSF taken from the same animals at the same time of day several days prior to dosing, while the control for cortex samples was cortex taken from non-treated monkeys.

Cynomolgus monkey brains were thawed and ~1.0 g of cortex was dissected and minced with a razor blade. The minced sample was then homogenized and extracted with guanidine buffer as described[2] except that homogenization was carried out in a 1.5 mL Eppendorf tube using a conical Teflon homogenizer (10 strokes) followed by sequential needle passage through 18 gauge and 23 gauge needles (8x each). CSF and cortical Aβ_{40} were measured in 25 μL of monkey plasma or CSF and 10 μL of cortical extract as described below.

b. Rhesus monkey plasma and CSF sample preparation

The cisterna magna ported (CMP) rhesus monkey[3] model and methods for CSF and plasma sampling from CMP rhesus monkeys[4] have previously been described. The study was a full crossover four period design with four-week washout between periods. Compound **9** was formulated in 90% PEG 400/10% Tween 80 vehicle. Conscious CMP rhesus monkeys (n = 6) received vehicle, or 10, 30, and 100 mg/kg **9** administered orally as a solution.

c. Measurement of monkey plasma and CSF Aβ and sAPPβ levels

Cynomolgus monkey CSF Aβ_{40} and rhesus monkey plasma and CSF Aβ_{40}, Aβ_{42} and sAPPβ levels were measured with commercially available, performance validated human immunoassays according to the manufacturer's instructions (MesoScale Diagnostics; Aβ_{40}, catalog No. K111FTE; Aβ_{42}, catalog No. K111FUE; and sAPPβ, catalog No. K111BTE). Human Aβ_{40}, Aβ_{42} and sAPPβ standards were used for calibration curves. The analytical ranges of quantitation were 62.5 to 15,000 pg/mL, 40.0 to 2,000 pg/mL, and 61.5 to 30,000 pg/mL for Aβ_{40}, Aβ_{42}, and sAPPβ, respectively. CSF samples were supplemented with Tween-20 at 0.5% final concentration to enhance recovery of Aβ.

d. Measurement of monkey cortex Aβ_{40} levels

Cynomolgus monkey cortical Aβ_{40} was measured in a Luminex®-based assay that utilized biotinylated-WO2 (b-WO2) antibody and G2-10 antibody[5] coupled to BioPlex COOH beads (BioRad). The assay was assembled by addition of 100 μL of 1x LAβ_{40} buffer (0.05 M HEPES [pH 7.5], 0.2% BSA, 0.2% Tween-20, 0.15 M NaCl) to each well followed by addition of 50 μL each of G2-10 coupled beads (1000 beads/well) and b-WO2 (0.5 μg/mL final concentration) in 1x LAβ_{40} buffer and 10 μL of cortical extract or human Aβ_{40} standards. Guanidine HCl was

added to the synthetic human Aβ_{40} standard curve to control for matrix effects. The assay was incubated overnight and wells were then cleared and washed twice with 100 μL of 1x LAβ_{40} buffer. Fifty μL of phycoerythrin-conjugated streptavidin (PE-streptavidin, BioRad) diluted 100-fold in 1x LAβ_{40} buffer was added to each well and incubated for 1 hour at room temperature with shaking. Unbound PE-streptavidin was removed by three 100 μL washes with cold cytokine assay buffer (BioRad). Washed beads were suspended in 125 μL of cold cytokine assay buffer by shaking on a microplate shaker. Plates were read on a BioPlex suspension array system (BioRad) with target region beads set to 40 beads/region and the upper end of the DD gate set to 10,000. Raw fluorescence data was analyzed using nonlinear regression analysis and absolute Aβ_{40} levels were extrapolated from the standard curve using GraphPad Prism 4.0.2.

References

1. Mandal, M.; Zhu, Z.; Cumming, J. N.; Liu, X.; Strickland, C.; Mazzola, R. D.; Caldwell, J. P.; Leach, P.; Grzelak, M.; Hyde, L.; Zhang, Q.; Terracina, G.; Zhang, L.; Chen, X.; Kuvelkar, R.; Kennedy, M. E.; Favreau, L.; Cox, K.; Orth, P.; Buevich, A.; Voigt, J.; Wang, H.; Kazakevich, I.; McKittrick, B. A.; Greenlee, W.; Parker, E. M.; Stamford, A. W. Design and validation of bicyclic iminopyrimidinones as beta amyloid cleaving enzyme-1 (BACE1) inhibitors: conformational constraint to favor a bioactive conformation. *J. Med. Chem*. **2012**, *55*, 9331-9345.
2. Stamford, A. W.; Scott, J. D.; Li, S. W.; Babu, S.; Tadesse, D.; Hunter, R.; Wu, Y.; Misiaszek, J.; Cumming, J. N.; Gilbert, E. J.; Huang, C.; McKittrick, B. A.; Hong, L.; Guo, T.; Zhu, Z.; Strickland, C.; Orth, P.; Voigt, J. H.; Kennedy, M. E.; Chen, X.; Kuvelkar, R.;

Hodgson, R.; Hyde, L. A.; Cox, K.; Favreau, L.; Parker, E. M.; Greenlee, W. J. Discovery of an orally available, brain penetrant BACE1 inhibitor that affords robust CNS Aβ reduction. *ACS Med. Chem. Lett*. **2012**, *3*, 897-902.

3. Gilberto, D. B.; Zeoli, A. H.; Szczerba, P. J.; Gehret. J. R.; Holahan, M. A.; Sitko, G. R.; Johnson, C. A.; Cook, J. J.; Motzel, S. L. An alternative method of chronic cerebrospinal fluid collection via the cisterna magna in conscious rhesus monkeys. *Contemp. Top Lab. Anim. Sci.* **2003**, *42*, 53-59.
4. Sankaranarayanan, S.; Holahan, M. A.; Colussi. D.; Crouthamel, M. C.; Devanarayan, V.; Ellis J.; Espeseth, A.; Gates, A. T.; Graham, S. L.; Gregro. A. R. ; Hazuda, D.; Hochman, J. H.; Holloway, K.; Jin, L.; Kahana, J.; Lai, M. T.; Lineberger, J.; McGaughey, G.; Moore, K. P.; Nantermet, P.; Pietrak, B.; Price, E. A.; Rajapakse, H.; Stauffer, S.; Steinbeiser, M. A.; Seabrook, G.; Selnick, H. G.; Shi, X. P.; Stanton, M. G.; Swestock, J.; Tugusheva, K.; Tyler, K. X.; Vacca, J. P.; Wong, J.; Wu, G.; Xu, M.; Cook, J. J.; Simon, A. J. First demonstration of cerebrospinal fluid and plasma Aβ lowering with oral administration of a β-site amyloid precursor protein-cleaving enzyme 1 inhibitor in nonhuman primates. *J. Pharmacol. Exp. Ther.* **2009,** *328*, 131–140.
5. Ida, N.; Hartmann, T.; Pantel, J.; Schroder, J.; Zerfass, R.; Forstl, H.; Sandbrink, R.; Masters, C.L.; Beyreuther, K. Analysis of heterogeneous A4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. *J. Biol. Chem.* **1996**, *271*, 22908-22914.