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Letter

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# Discovery of an Orally Available, Brain Penetrant BACE1 Inhibitor That Affords Robust CNS Aβ Reduction

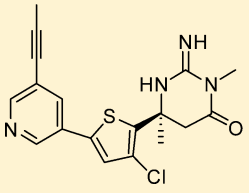
Andrew W. Stamford,*[,†,△] Jack D. Scott,[†] Sarah W. Li,[†] Suresh Babu,[‡] Dawit Tadesse,[‡] Rachael Hunter,[‡] Yusheng Wu,[†] Jeffrey Misiaszek,[†] Jared N. Cumming,[†] Eric J. Gilbert,[†] Chunli Huang,[†] Brian A. McKittrick,[†] Liwu Hong,[†] Tao Guo,[‡] Zhaoning Zhu,[†] Corey Strickland,[#] Peter Orth,[#] Johannes H. Voigt,[#] Matthew E. Kennedy,[§] Xia Chen,[§] Reshma Kuvelkar,[§] Robert Hodgson,[§] Lynn A. Hyde,[§] Kathleen Cox,[±] Leonard Favreau,[±] Eric M. Parker,[§] and William J. Greenlee[†]

[†]Departments of Medicinal Chemistry, [§]Neurobiology, [±]Drug Metabolism, and [#]Global Structural Chemistry, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033, United States

[‡]Ligand Pharmaceuticals, Inc., 3000 Eastpark Boulevard Cranbury, New Jersey 08512, United States

Supporting Information

**ABSTRACT:** Inhibition of BACE1 to prevent brain Aβ peptide formation is a potential disease-modifying approach to the treatment of Alzheimer's disease. Despite over a decade of drug discovery efforts, the identification of brain-penetrant BACE1 inhibitors that substantially lower CNS Aβ levels following systemic administration remains challenging. In this report we describe structure-based optimization of a series of brain-penetrant BACE1 inhibitors derived from an iminopyrimidinone scaffold. Application of structure-based design in tandem with control of physicochemical properties culminated in the discovery of compound **16**, which potently reduced cortex and CSF Aβ40 levels when administered orally to rats.




**16**

BACE1 $K_i$: 1.7 nM
cell Aβ40 $IC_{50}$: 11 nM
rat cortex Aβ40 $ED_{50}$: 6 mg/kg p.o.
rat CSF Aβ40 $ED_{50}$: 4 mg/kg p.o.

**KEYWORDS:** *BACE1, inhibitor, Alzheimer's disease, Aβ40, iminopyrimidinone, X-ray crystallography*

Alzheimer's disease (AD) is a chronic neurodegenerative disease and is now the sixth leading cause of death in the Unites States.[1] Current treatments of AD are palliative and only transiently effective, and they do not impact disease progression.[2] Due to the progressive and debilitating nature of the disease and the lack of disease-modifying therapeutic agents, novel treatments that slow or halt disease progression are urgently sought.

Neuropathological hallmarks of AD are the accumulation of β-amyloid as extracellular neuritic plaques and the appearance of intracellular neurofibrillary tangles comprised of hyperphosphorylated tau protein.[3] Although the cause of AD remains poorly understood, several lines of evidence suggest that accumulation of β-amyloid is an underlying cause of neuronal cell death.[3−5] The amyloid peptides are produced from amyloid precursor protein (APP) in two proteolytic steps, the first of which is mediated by the aspartyl protease β-site APP cleaving enzyme-1 (BACE1)[6] to produce a C-terminal fragment that is subsequently processed by γ-secretase to yield Aβ peptides of 36−43 amino acids. While Aβ40 is the most abundant species formed, Aβ42 is more prone to aggregation and formation of oligomeric neurotoxic species.[7−9] BACE1 inhibition to prevent Aβ peptide formation is considered to be a highly attractive amyloid-lowering approach to a potentially disease-modifying AD treatment. In this regard, BACE1 knockout mice do not produce β-amyloid, demonstrating that BACE1 is necessary for Aβ production *in vivo*.[10−12] Furthermore, BACE1 knockout mice do not exhibit overt abnormalities, displaying a moderate peripheral nerve hypomyelination caused by loss of BACE1-mediated processing of Neuregulin-1.[13,14]

For more than a decade, there have been intense efforts to identify potent, selective BACE1 inhibitors that effectively inhibit Aβ peptide production in the CNS of preclinical animal models. Although substantial work has been carried out on peptidomimetic inhibitors, this has met with limited success, as such inhibitors tend to be Pgp substrates and suffer restricted brain penetration.[15] Nonpeptidic BACE1 inhibitors have been reported by us[16−18] and others,[19−23] and they offer the most promising avenue to the discovery of orally bioavailable, brain penetrant inhibitors. A recent report of a nonpeptidic BACE1 inhibitor that produced substantial reductions in central Aβ levels in preclinical species and in healthy volunteers confirms the validity of this approach.[24]

We had previously reported the discovery of the potent iminohydantoin BACE1 inhibitor **1** that was rationally designed from fragment-based screening hits.[16,17] In the course of lead optimization, the identification of **1** was an important milestone en route to the discovery of the highly ligand efficient

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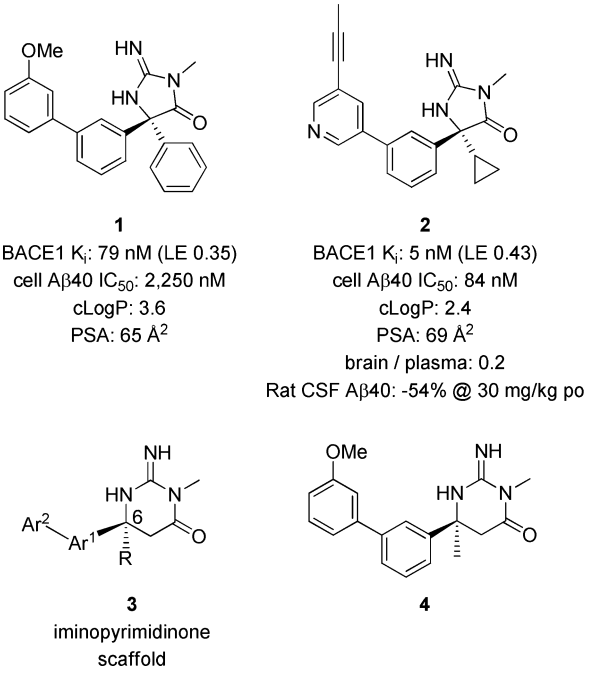
iminohydantoin BACE1 inhibitor **2** (Chart 1).[18] Upon oral administration to rats at the dose of 30 mg/kg, **2** acutely

**Chart 1. Iminoheterocycle BACE1 Inhibitor Design**




lowered CSF A$\beta$40 by approximately 50% 3 h postdose relative to vehicle-treated controls. While the ability of **2** to elicit CSF A$\beta$ reduction represented a significant advance in our efforts to develop centrally active BACE1 inhibitors, **2** had a significant $IC_{50}$ shift in a cell-based A$\beta$40 assay relative to its BACE1 $K_i$ and limited brain penetration. Consequently, in order to develop inhibitors with improved *in vivo* potency, concurrent optimization efforts focused on the identification of inhibitors with improved enzymatic/cellular potency shifts and brain exposures. Toward this goal, we report herein the design and optimization of a series of BACE1 inhibitors based on the homologous iminopyrimidinone scaffold **3** that also evolved from the lead iminohydantoin **1** (Chart 1).

An X-ray cocrystal structure of **1** bound to BACE1 revealed that the guanidine functionality of **1** forms intricate H-bond donor−acceptor interactions with $Asp^{32}$ and $Asp^{228}$ at the BACE1 active-site, the methoxybiphenyl moiety occupies the contiguous S1 and S3 pockets, and the remaining phenyl substituent occupies space in the vicinity of S2′ (Figure 1). In

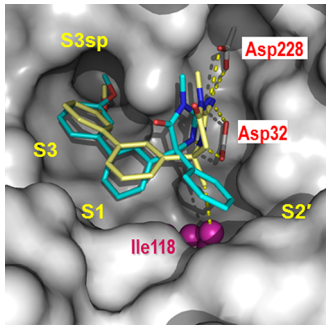



**Figure 1.** X-ray cocrystal structure of BACE1 and **1** (blue) superimposed with docked **4** (yellow).

order to maximize the probability of identifying inhibitors with high CNS exposure, our optimization strategy was to adhere to the design principles of maintaining or improving LE while limiting MW, restricting cLogP in a desirable range (≤4), and avoiding additional H-bond donors to minimize Pgp activity and polar surface area.[25] In accordance with this approach, we pursued focused modifications of the iminoheterocyclic core and of the biaryl motif that occupies the S1 and S3 pockets of the enzyme.

Ring expansion of the core to a six-membered iminopyrimidinone **3** was expected to have a significant impact on physicochemical properties and conformation. In comparison to the iminohydantoin core, the iminopyrimidinone is predicted to be more basic.[26] We reasoned that enhanced basicity could increase cell potency by favoring partitioning to the acidic intracellular compartment where BACE1-mediated APP processing is operative. A second major design consideration centered on conformational differences between the five- and six-membered ring iminoheterocycle scaffolds. The iminopyrimidinone ring differs from the planar iminohydantoin core in that the additional $sp^3$ center imparts a significant pucker to the ring. Consequently, the docked pose of the iminopyrimidinone **4** (Chart 1) overlaid with iminohydantoin **1** places the S1 phenyl substituent and the amidine in a similar location; however, the 6-methyl substituent has a trajectory that diverges from that of its iminohydantoin counterpart, projecting toward $Ile^{118}$ (Figure 1).[27] Based on this analysis, it was hypothesized that substituents at C6 smaller than phenyl projecting toward the prime-site might be necessary in order to avoid unfavorable steric interactions with the enzyme.

To test these hypotheses, targeted sets of iminopyrimidinones **4**−**6** and **7**−**9** were prepared that differed in their substitution at C6 of the iminopyrimidinone ring (Table 1). As predicted by docking experiments, the 6-methyl iminopyrimidinones **4**−**6** had BACE1 affinities that were substantially higher than those of their 6-phenyl counterparts **7**−**9** and were comparable to that of the iminohydantoin **1**. Also noteworthy was the observation that the cell A$\beta$40 $IC_{50}$ values of the iminopyrimidinones **4**−**6** were minimally shifted (2−3-fold) relative to their $K_i$ values, in contrast to the cases of the corresponding 5,5-diphenyl iminohydantoins represented by **1**.[18] Comparison of iminopyrimidinones **4**−**6** in a rat oral pharmacokinetic screen demonstrated that cyanophenyl derivative **5** had superior rat oral pharmacokinetic properties. Compound **5** adminstered at a dose of 10 mg/kg afforded a plasma $AUC_{0-6\ h}$ of 8.6 $\mu$M·h compared to corresponding values of <1 $\mu$M·h for **4** and **6** administered at the same dose.[28]

An X-ray cocrystal structure of the 6-methyl iminopyrimidinone derivative **4** and BACE1 was in close agreement with the predicted binding mode (Figure 2).[29] Notably, the bound conformation of the iminopyrimidinone ring projects the C6 methyl group to within 3.6 Å of $Ile^{118}$, consistent with a favorable hydrophobic interaction. As expected, the biaryl motif of **4** binds in the contiguous S1−S3 pockets, with the methoxyl group projecting toward the S3 subpocket. Well-defined density for the flap was observed, with the side-chain of $Tyr^{71}$ positioned <4 Å away from the methylene of the iminopyrimidinone core.

Given the encouraging results obtained with the 6-methyl iminopyrimidinones **4**−**6**, we next explored a wide range of biaryl and heterobiaryl motifs that could bind in S1−S3. The most productive of these was replacement of the proximal


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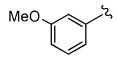
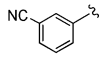
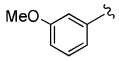
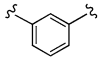
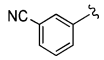
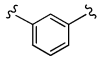
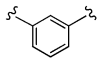
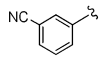
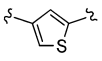
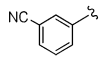
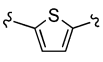
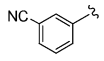
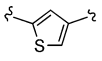
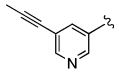
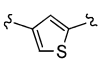
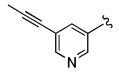
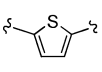
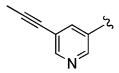
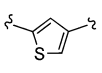
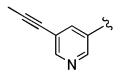
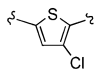
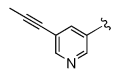
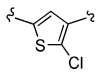
Table 1. BACE1 Affinity, Cell Activity, and Selectivity of Iminopyrimidinone Derivatives 3

| cpd | Ar² | Ar¹ | R | BACE1 $K_i$ nM[a] | LE | Cell Aβ40 $IC_{50}$ nM[a] | CatD/BACE1[b] |
|---|---|---|---|---|---|---|---|
| 4 |  |  | Me | 270 | 0.22 | 840 | 61 |
| 5 |  |  | Me | 350 | 0.24 | 450 | 63 |
| 6 |  |  | Me | 670 | 0.22 | 460 | 88 |
| 7 |  |  | Ph | 22,000 | 0.37 | nd[c] | 0.1 |
| 8 |  |  | Ph | 9,500 | 0.37 | nd[c] | 0.7 |
| 9 |  |  | Ph | 44,000 | 0.38 | nd[c] | 0.6 |
| 10 |  |  | Me | 57 | 0.43 | 68 | 68 |
| 11 |  |  | Me | 88 | 0.42 | 120 | 26 |
| 12 |  |  | Me | 210 | 0.40 | 400 | >25 |
| 13 |  |  | Me | 7.8 | 0.46 | 13 | 130 |
| 14 |  |  | Me | 33 | 0.42 | 42 | 47 |
| 15 |  |  | Me | 47 | 0.42 | 110 | 42 |
| 16 |  |  | Me | 1.7 | 0.48 | 11 | 21 |
| 17 |  |  | Me | 8.0 | 0.44 | 67 | 12 |

[a]BACE1 $K_i$ and cell $IC_{50}$ values are the average of a minimum of two independent determinations. [b]Selectivity ratios were derived from cathepsin D $K_i$ values (average of a minimum of two independent determinations). [c]Not determined.

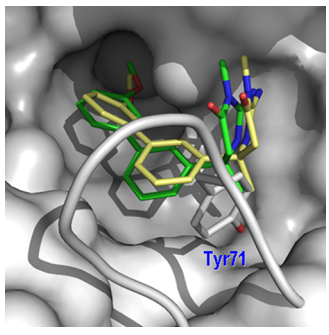



**Figure 2.** Comparison of **4** (yellow) docked to BACE1 and the X-ray cocrystal structure of **4** (green).

phenyl ring with the bioisosteric thiophenyl group. In exploring this modification, distal (3-cyano)phenyl substitution was chosen because of the superior pharmacokinetic properties conferred by this substituent. Of the three possible thiophenyl regioisomers **10**–**12** that maintain the iminopyrimidinone core and 3-cyanophenyl substituent in a 1,3-relationship, we were gratified to find that the affinities and LEs of the 2,4-thiophenyl and 2,5-thiophenyl regioisomers **10** and **11** were significantly improved compared to those of the corresponding biphenyl derivative **5** (Table 1). This improvement in affinity also translated to improved cell potency, with the 2,4 regioisomer **10** ($K_i$ 57 nM; cell $IC_{50}$ 68 nM) exhibiting $K_i$ and cell $IC_{50}$ values of <100 nM.

Replacement of the 3-cyanophenyl moiety by the 3-prop-1-ynyl-5-pyridyl group as in **2**, analogs **13**–**15**, resulted in further improved BACE1 affinity and cell activity, with **13** ($K_i$ 7.8 nM; cell $IC_{50}$ 13 nM) achieving single-digit nanomolar affinity and a cell potency shift of only 2-fold. A further gain of affinity could be realized by incorporation of chloro substitution at the thiophene 4-position of **14** to afford **16**, which had a $K_i$ value of 1.7 nM. Similar to the case of **16**, chloro substitution at the thiophene 5-position of **15** gave **17** with enhanced affinity. The increased lipophilicity conferred by chloro substitution resulted in only modestly increased cell potency shifts, with **16** maintaining good cell activity ($IC_{50}$ 11 nM).

The selectivities of the iminopyrimidinone inhibitors for BACE1 versus the closely related aspartyl protease cathepsin D ranged from 61- to 130-fold for the biphenyl derivatives **4**–**6** and the 2,4-thiophenyl analogs **10** and **13**, and they were generally somewhat decreased for the 2,5- and 3,5-thiophenyl analogs (e.g., **11**, **14**–**17**). In addition to selectivity over cathepsin D (21-fold; Table 1), **16** was profiled against other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human aspartyl proteases. Compound **16** is not selective for BACE1 over the close homologue BACE2 ($K_i$ < 1 nM) but exhibits good selectivity over cathepsin E (350×) and pepsin (260×) and excellent selectivity over renin (>10,000×).

An X-ray cocrystal structure showed that **16** bound to BACE1 in a mode highly consistent with **4**, but it was notable in two respects that may contribute to its higher affinity (Figure 3). First, the propynyl group of **16** binds deep in the S3

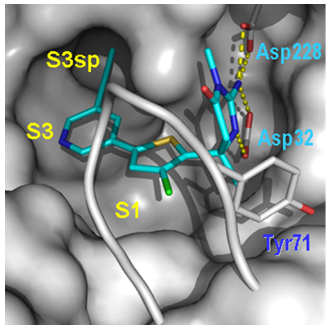



**Figure 3.** X-ray cocrystal structure of **16** (blue) and BACE1.

subpocket where it is positioned to engage in a hydrophobic interaction with Ala[335], as previously reported for **2**.[18] Second, in comparison to the cocrystal structure of **4**, the flap is in a more closed conformation, forming a pocket which accommodates the chloro substituent while positioning the side-chain of flap Tyr[71] in close proximity to the methylene of the iminopyrimidinone core.

In Caco-2 cells, **16** exhibited good permeability ($P_{app}$ 153 nm·s$^{-1}$) and a low efflux ratio (AP/BL 2.4), suggesting that the compound does not possess an overt human Pgp liability. In rat and human hepatocytes, **16** had low intrinsic clearance (4.9 and 2.7 μL·min$^{-1}$·10$^6$ cells, respectively), reflecting low metabolic turnover, and displayed significant free fractions of 0.02 and 0.04, respectively, in rat and human plasma protein binding assays. These *in vitro* parameters translated to favorable rat pharmacokinetic properties, with **16** displaying high oral bioavailability (69%) and plasma exposure, a plasma half-life of 2.8 h, and an average brain/plasma ratio of 3.

Based on its excellent *in vitro* and rat pharmacokinetic profiles, **16** was subjected to pharmacodynamic profiling in rats. In a dose titration study, **16** elicited a robust reduction in CSF and cortex Aβ40 levels with $ED_{50}$ values of 4 and 6 mg/kg in the respective compartments 3 h after oral administration (Figure 4). As shown in Figure 4, plasma and brain exposures increased in a relatively dose-proportional manner across the dose range of 0.3 to 30 mg/kg.

Optimization of **1** to inhibitor **16** achieved a significant gain in BACE1 affinity without concomitant increase in number of H-bond donors, cLogP, polar surface area, or MW, as reflected by the large increase in LE from 0.35 kcal·mol$^{-1}$ to 0.48 kcal·mol$^{-1}$. In addition to good solubility (200 μM, pH 7.4), the measured physical properties of **16**, including a p$K_a$ value of 7.5 and a log *D* value of 1.8, fall within CNS druglike ranges.[30]

The synthesis of **16** (Scheme 1) employed an Ellman asymmetric aldol condensation to assemble the β-amino acid subunit and set the stereochemistry of the quaternary center.[31] Thus, addition of the enolate of methyl acetate to (*R*)-*tert*-butylsulfinamide **19**, derived from 1-(3-chlorothiophen-2-yl)-ethanone **18**, in the presence of chlorotitanium triisopropoxide afforded the (*S*)-β-aminoester derivative **20** in a >99:1

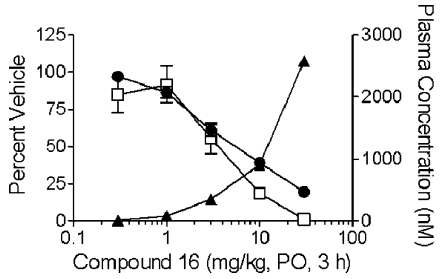
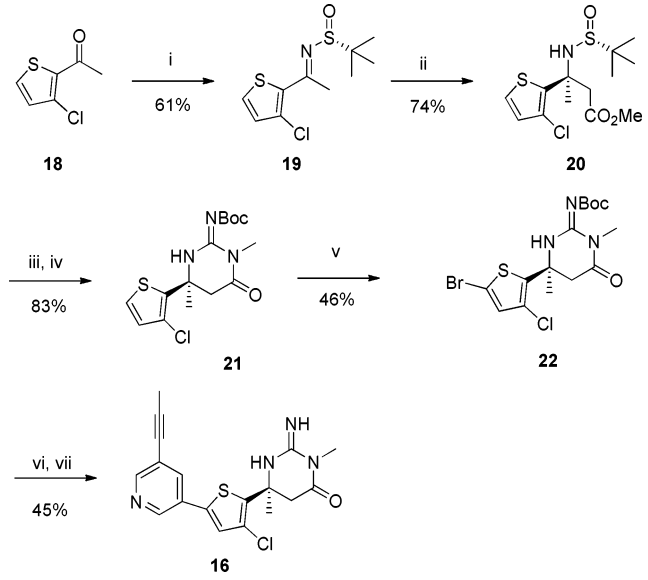



**Figure 4.** Dose response of **16** on CSF (□; collected from the cisterna magna) and cortex (●; Aβ40 3 h postdose in Sprague–Dawley rats (PO, formulated in 20% hydroxypropyl-β-cyclodextrin, 5 mL/kg). Doses ≥3 mg/kg were significantly different from vehicle, $p$ < 0.02, Dunnett's posthoc test. Dose dependent increase in plasma concentration (▲) of **16**; brain/plasma ratios ranged from 2.4 to 4.2.

**Scheme 1. Synthesis of Compound 16**[a]




[a]Reagents and conditions: (i) (*R*)-2-methylpropane-2-sulfinamide, Ti(OEt)$_4$, THF, reflux; (ii) LDA, methyl acetate, ClTi(O*i*Pr)$_3$, THF, −78 °C; (iii) HCl, 1,4-dioxane; (iv) EDCI, *N*-[(methylamino)-thioxomethyl]-*tert*-butylcarbamate, *i*Pr$_2$NEt, THF; (v) NBS, DMF, 50 °C; (vi) (5-(prop-1-ynyl)pyridin-3-yl)boronic acid, 2 M Na$_2$CO$_3$, PdCl$_2$(dppf)·CH$_2$Cl$_2$, 1,4-dioxane, 65 °C; (vii) CF$_3$COOH, CH$_2$Cl$_2$.

diastereomeric ratio. Acidic cleavage of the sulfinamide of **20** and coupling of the derived amine to *tert*-butyl *N*-[(methylamino)-thioxomethyl]carbamate proceeded with concomitant intramolecular cyclization to give the iminopyrimidinone derivative **21**. Bromination of the thiophene afforded **22**, which was subjected to sequential Suzuki coupling with (5-(prop-1-ynyl)pyridin-3-yl)boronic acid and Boc deprotection to give **16**.

In conclusion, application of structure-based design with simultaneous control of physicochemical parameters enabled discovery of the iminopyrimidinone BACE1 inhibitor **16** starting from prototype iminohydantoin **1**. The robust reduction of CNS Aβ levels that was achieved following oral administration of **16** to rats demonstrates the potential of the iminoheterocycle class of BACE1 inhibitors to deliver agents that could test the therapeutic utility of BACE1 inhibition in clinical trials.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## ■ ASSOCIATED CONTENT

### Supporting Information

Synthetic methods and characterization data for compounds **1**–**17**, and methods for *in vitro*, *in vivo*, and pharmacokinetic assays. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION


### Corresponding Author

*Tel: 732-594-1960. E-mail: andrew.stamford@merck.com.

### Present Address

△Merck Research Laboratories, 126 E. Lincoln Ave., Rahway, NJ 07065, United States.


### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

AD, Alzheimer's disease; APP, amyloid precursor protein; BACE1, β-site APP cleaving enzyme-1; CNS, central nervous system; CSF, cerebrospinal fluid; Pgp, P-glycoprotein; LE, ligand efficiency; CatD, cathepsin D

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