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Design of an orally efficacious hydroxyethylamine (HEA) BACE-1 inhibitor in a preclinical animal model

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ABSTRACT

In this Letter, we describe our efforts to design HEA BACE-1 inhibitors that are highly permeable coupled with negligible levels of permeability-glycoprotein activity. These efforts culminate in producing **16** which lowers Aβ by 28% and 32% in the cortex and CSF, respectively, in the preclinical *wild type* Hartley guinea pig animal model when dosed orally at 30 mpk BID for 2.5 days.

Alzheimer's disease (AD) is a form of senile dementia, characterized by a progressive loss of memory and cognitive ability, affecting more than 35 million elderly people worldwide with skyrocketing healthcare costs far exceeding $100 billion annually.[1] The pathology of this neurodegenerative disorder uniquely manifests itself with the presence of extraneuronal aggregation of plaques composed of β-amyloid peptides (Aβ).[2] Intracellular neurofibrillary tangles (NFTs), aggregates of aberrantly hyperphosphorylated tau proteins, are also a component of the pathology but not exclusive to AD.[3] Aβ-peptides are derived from the sequential proteolytic cleavage of the β-amyloid precursor protein (β-APP) by two aspartic acid proteases, referred to as β- and γ-secretase, respectively.[4] Inhibition of either protease[5] has been demonstrated to result in reduction of brain Aβ-peptide in preclinical studies.[6] Inhibitors of either protease offer attractive candidates as potentially disease-modifying, rather than palliative, treatments for people afflicted with this debilitating malady.[7]

Between these two proteases, β-secretase (BACE-1) may be a more alluring therapeutic target based on the following distinctions. γ-Secretase processes a myriad of substrates,[8] such as Notch, raising concerns about mechanism-based side-effects due to a deficiency in selectivity.[9] Conversely, gene deletion of BACE-1 in mice produced no consistent phenotypic differences between their *wild type* counterparts.[10] These knockout mice are without compensatory activity, thus devoid of the ability to generate Aβ in the brain.[11] Modest reductions of BACE-1 activity resulted in significant reduction of plaque burden.[12] Furthermore, cleavage of β-APP by BACE-1 is the rate-limiting step in Aβ-peptide production[4] and releases the soluble N-terminal APP fragment which initiates a cascade event ultimately leading to apoptosis.[13] Additionally, soluble oligomeric Aβ42 indirectly signals for hyperphosphorylation of tau at AD-specific epitopes producing NFTs that lead to neurotoxicity.[14] Moderately abating the production of Aβ monomers results in a disproportionate reduction of synaptotoxic soluble Aβ oligomers[15] leading to improved long-term potentiation,[16] a measure of memory and synaptic plasticity. Some reports suggest that the severity of cognitive deficits in AD patients

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correlates better with brain levels of soluble Aβ than fibrillized Aβ.[17] These data validates BACE-1 and presents its clear advantages over γ-secretase as a therapeutic target, and buttresses the amyloid cascade hypothesis.[18]

Previously, we reported our research concerning the *S1′*[19a] and *S2′*[19b] pockets as well as the aryl linker[19c] of our hydroxyethylamine (HEA) peptidomimetics. Permeability-glycoprotein (P-gp) efflux activity has been the dominant impediment for the HEA template to penetrate into the parenchyma of the brain and lower Aβ. Herein, we will describe the properties of the HEAs that led to our working hypothesis concerning the pharmacokinetic deficiencies, and the strategy to design highly permeable BACE-1 inhibitors devoid of P-gp liability. The terminology used to classify the permeability and recovery of the compounds is outlined in Table 1. The recovery is a measure of the inhibitors affinity to embed in the membrane (low recovery equates to high membrane affinity) vide infra.

The binding mode of our HEA BACE-1 inhibitors is as follows: the difluoroaryl, cyclohexyl, and *tert*-butyl substituents occupy the *S1*, *S1′*, and *S2′* pockets, respectively (Fig. 1).[19] The protonated amine and hydroxyl group binds at the scissile site to Asp32 and Asp228, respectively. The carbonyl of Gly34 forms a hydrogen bond with the ionized amine and the hydrogen on the aryl linker flanked by the two alkyl groups.[20] The acetamide functions as a bi-dentate ligand with the carbonyl forming a hydrogen bond with the flap residue Gln73 while the NH interacts with Gly230.

Compound **1**, while potent, suffers from poor permeability (7 nm/s) and low recovery (11%) in the in vitro Madin-Darby canine kidney (MDCK) cell assay (Table 2). Highly lipophilic molecules that possess a basic amine exhibit a pronounced affinity towards membranes.[21] The measured log *P* and pK_a of **1** is 4.8 and 8.1, respectively. Our working hypothesis involved the basic amine binding to the polar head and the greasy portion partitioning into the lipophilic tail of the membrane (Fig. 2). To overcome this liability, we sought to lower the log *P* on the prime side and modulate the pK_a of the amine below the pH (7.4) of the medium. The *P1′* pyran **2** has a measured log *P* of 4.3 and pK_a of 7.2 which imparts a moderate rate of permeability and an improved recovery which indicates a reduction in membrane affinity. The pyrimidine **3** has a log *P* and pK_a of 3.7 and 6.7, respectively, and these features led to excellent permeability and recovery with heterocyclic aryl linkers in general. Similarly, a pattern of increased permeability and recovery arose with a polar *P2′* (**4** and **5**). Another trend emerged when a polar *P1′* combined with a polar *P2′*, **6** and **7**, led to reduced permeability, relative to **2**–**5**, in conjunction with high levels of recovery implying that compounds of this archetype had capitulated membrane affinity. Elevated P-gp efflux activity was also observed for **6**. These results indicated that a fine balance of physicochemical properties was necessary to penetrate the blood brain-barrier (BBB). P-gp efflux activity remained a significant obstacle as it was unaffected or exacerbated by these perturbations.

Compound **4** was examined in vivo for correlation with the in vitro assays. Hartley guinea pigs, a *wild type* preclinical animal model, were dosed orally up to 100 mpk. At the highest dose no reduction of central Aβ was detected while the plasma Aβ levels were reduced by 82%. Inspection of the time course profile of the brain and plasma levels of **4** (Fig. 3) revealed a peculiarity: the brain levels did not track with the plasma levels, but instead remained constant throughout the study. This implied that **4** embedded into the extensive network of endothelial cells that constitute the BBB,[22] and did not penetrate into the parenchyma

Table 1
Classification of permeability and recovery

Class	P_{app} (nm/s)	% recovery
Poor	⩽25 nm/s	⩽10%
Low	26 nm/s ⩽ 50 nm/s	11% ⩽ 30%
Moderate	51 nm/s ⩽ 99 nm/s	31% ⩽ 50%
Good	—	51% ⩽ 70%
High	⩾100 nm/s	⩾71%

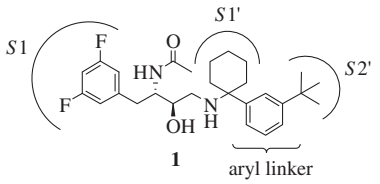
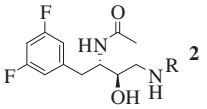
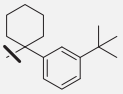
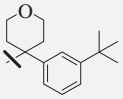
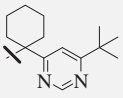
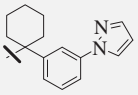
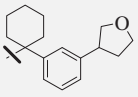
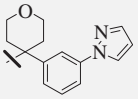
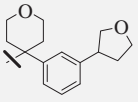


Figure 1. Binding mode of the HEAs.

Table 2
SAR of the cyclohexyl prime side binding region[a]



#	R	BACE-1 IC_{50} (nM) cell ED_{50} (nM) Cat-D IC_{50} (nM)	P_{app} (nm/s) % recovery P-gp efflux ratio	log *P* @ pH =7.4 pK_a
1		47 17 25	7 nm/s 11% 19	4.8 8.1
2		61 15 230	81 nm/s 46% 18	4.3 7.2
3		2400 350 250	265 nm/s 112% 20	3.7 6.7
4		59 4.0 22	72 nm/s 54% 22	4.0 7.9
5[b]		88 7.1 140	100 nm/s 64% 31	4.0 8.1
6		110 13 160	48 nm/s 77% 48	2.9 na
7[b]		290 28 150	12 nm/s 77% na	2.8 na

[a] See Ref. 19a for experimental and synthetic details.
[b] 1:1 mixture of epimers.

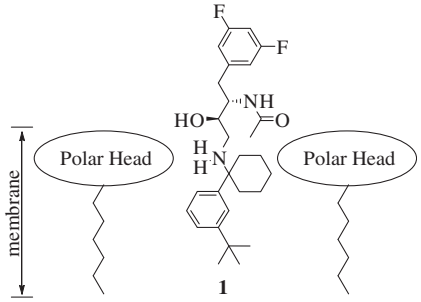


Figure 2. Schematic of proposed HEA anchoring into the cell membrane.

of the brain despite the improvements to both the permeability and recovery in the in vitro MDCK assay. The desired profile of the inhibitor should have the brain levels mirror those of the plasma, an in-and-out profile.

A search for compounds with an in vivo in-and-out profile and a method to ameliorate the P-gp liability was initiated. Modulating the pK_a of the amine on the HEA template to reduce the P-gp liability was critical.[6a,19b,23] A *P1′* cyclopropyl was used to attenuate the basicity of the amine in place of heteroatoms which induced P-gp activity. Compound **10** had a low rate of forward flux (50 nm/s) and recovery (27%) indicating a high degree of membrane affinity (Table 3). Interestingly, **10** had a negligible P-gp efflux ratio of 1.3. This demonstrated that the HEA template was not inherently flawed with insurmountable P-gp liability. Employing the previous tactics to create permeability through the use of a polar aryl linker or *P2′* once again led to high rates of permeability with good to high levels of recovery, but untenable levels of P-gp efflux remained albeit at significantly depressed levels relative to their cyclohexyl counterparts. A gambit to create polarity without using heteroatoms was needed. From the viewpoint that polarity was simply a dipole moment, then halogens could be utilized to create polarity and do so in a manner that P-gp would be unable to recognize. Fluorine reduces the log *P* of and generates a dipole moment on hydrocarbons.[24] To expedite the testing of the hypothesis a readily accessible fluorinated *P2′*, **14**, was prepared via a Suzuki-Miyaura cross-coupling reaction[25] between 3-cyanophenylboronic acid and 2-bromo-3,3,3-trifluoroprop-1-ene followed by chemistry analogous to that of Scheme 1. Gratifyingly, **14** afforded a high rate of permeability (103 nm/s) with good recovery (66%), and most importantly, **14** was devoid of P-gp liability. Increased enzymatic activity against BACE-1 could be attained by increasing the branching at the benzylic position of the *P2′* motif.[19b] Furthermore, molecular modeling indicated the possibility of the newly introduced *P2′* fluoride(s) forming a hydrogen bond with the side chain of the Arg128 and/or Tyr198 which could lead to an improvement in potency relative to **10**.[20] To this end, several fluoroalkyls **15**–**17** were synthesized (Schemes 1–3) to probe for an increase in potency. As expected, these perturbations (**15**–**17**) enhanced the potency in comparison to **14**, maintained high rates of permeability coupled with good to high levels of recovery, and had diminutive levels of P-gp efflux activity (1.1–1.7).

The carbon framework of the *P2′* was installed via a palladium catalyzed enolate cross-coupling[26] between **20** and **21** (Scheme 1).

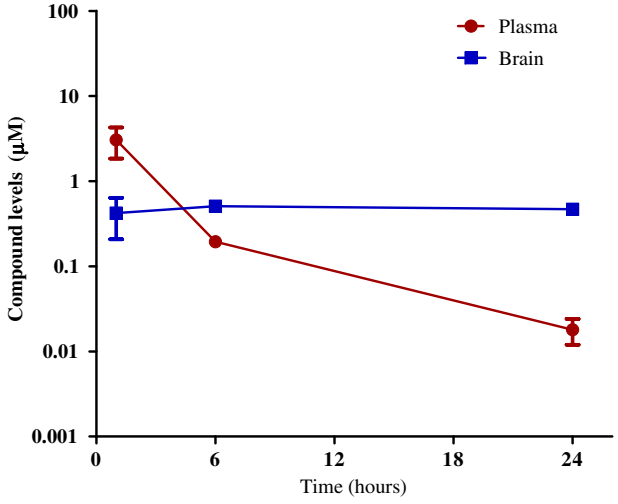
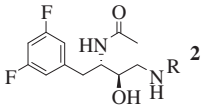
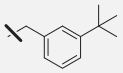
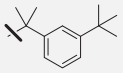
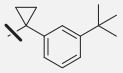
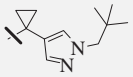
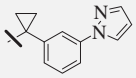
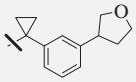
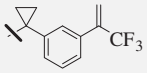
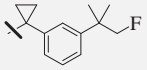
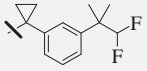
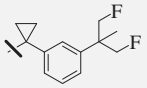
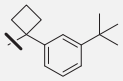
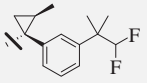


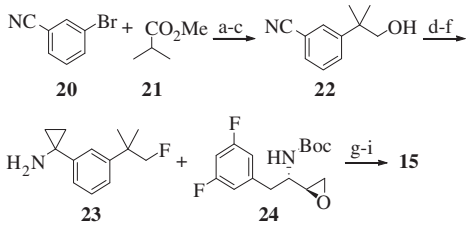
Figure 3. Twenty-four hour time course profile of the brain (blue) and plasma (red) levels of **4** after oral dosing in guinea pigs at 100 mpk.

Table 3
SAR of the prime side binding region[a]



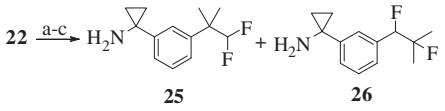
#	R	BACE-1 IC_{50} (nM) cell ED_{50} (nM) Cat-D IC_{50} (nM)	P_{app} (nm/s) % recovery P-gp efflux ratio	log *P*@ pH = 7.4 pK_a
8		1300 84 >10,000	13 nm/s 25% na	4.1 na
9		200 17 610	7 nm/s 20% na	4.4 na
10		140 51 220	50 nm/s 27% 1.3	4.6 6.6
11		350 54 720	102 nm/s 67% 11	3.0 7.1
12		980 230 750	143 nm/s 67% 6.0	3.1 6.7
13[b]		760 97 480	132 nm/s 71% 8.5	3.1 na
14		1300 620 1200	103 nm/s 66% 0.78	4.6 na
15		290 25 480	186 nm/s 64% 1.7	4.1 na
16		230 26 490	122 nm/s 54% 1.1	4.0 6.7
17		390 21 500	168 nm/s 78% 1.5	3.7 na
18		610 110 380	3 nm/s 17% na	4.6 7.8
19[c]		1700 130 na	25 nm/s 26% 2.6	4.4 na

[a] See Ref. 19a for experimental details.
[b] 1:1 mixture of epimers.
[c] Most active of the four diastereomers.

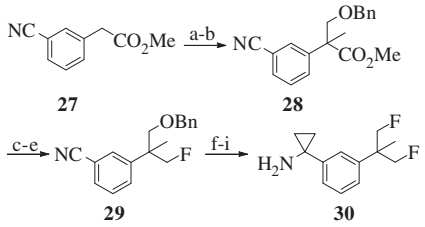


Scheme 1. Reagents and conditions: (a) *n*-BuLi, Cy_2NH, Pd_2dba_3–$CHCl_3$, P(*tert*-Bu)$_3$-HBF_4, PhMe, 58%; (b) aq NaOH, dioxane, MeOH; (c) BH_3-DMS, THF, 80% over two steps; (d) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (e) TBAF, MeCN, 80 °C, 23% over two steps; (f) Ti(O-*i*Pr)$_4$, EtMgBr, Et_2O, 0 °C to ambient temperature then BF_3-OEt_2, 48%; (g) *N*,*N*-diisopropylethylamine, isopropanol, reflux, 50%; (h) 4 N HCl in dioxane; (i) Ac_2ONMe, triethylamine, CH_2Cl_2, 65% over two steps.

Saponification of the ester followed by borane reduction gave the alcohol **22**. A two-step transformation of the alcohol into a fluoride followed by a modified Kulinkovich reaction[27] on the nitrile



Scheme 2. Reagents and conditions: (a) DMP, $NaHCO_3$, CH_2Cl_2, 72%; (b) DAST, CH_2Cl_2, 62%; (c) Ti(O-*i*Pr)$_4$, EtMgBr, Et_2O, 0 °C to ambient temperature then BF_3-OEt_2, 63% **25**:**26** 1:2.



Scheme 3. Reagents and conditions: (a) LiHMDS, MeI, THF, −78 °C, 91%; (b) NaHMDS, BOM-Cl, THF, −78 °C, 82%; (c) $LiBH_4$, THF, 40 °C, 82%; (d) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (e) TBAF, MeCN, 80 °C, 77% over two steps; (f) $FeCl_3$, CH_2Cl_2, 91%; (g) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (h) TBAF, MeCN, 80 °C, 63% over two steps; (i) Ti(O-*i*Pr)$_4$, EtMgBr, Et_2O, 0 °C to ambient temperature then BF_3-OEt_2, 55%.

furnished the cyclopropylamine **23**. Epoxide ring opening of **24**[28] with the amine **23**, deprotection, and acetylation[29] of the primary amine afforded **15**.

The alcohol **22** was oxidized with Dess–Martin periodinane[30] followed by treatment of the aldehyde with DAST (Scheme 2). The nitrile was converted to the cyclopropyl amine **25** via the modified Kulinkovich reaction. The cyclopropylamine **26** originated from a rearrangement during the DAST reaction as the major product. The aforementioned chemistry (vide supra) was employed to complete the synthesis of **16**.[31]

Sequential alkylation of **27** with iodomethane and BOM-Cl afforded **28** (Scheme 3). Reduction of the ester yielded the alcohol, which was converted to the fluoride **29**. Debenzylation of **29** with ferric chloride[32] afforded the alcohol that was transformed into a fluoride followed by conversion of the nitrile into the cyclopropylamine **30**. Standard chemistry (vide supra) was used to finish the synthesis of **17**.

The geminal di-fluoride **16** was administered orally at 10, 30, and 100 mpk to *wildtype* Hartley guinea pigs. No acute reduction of cortical Aβ was observed at 10 mpk, but a modest 12% and robust 42% reduction of cortical Aβ was observed at 30 and 100 mpk, respectively, 6 h post administration. Analysis of the time course profile of **16** revealed that the brain levels did not mirror those of the plasma, but they did decrease over time giving rise to a fast-in-slow-out profile (Fig. 4). Some portion of **16** was quite likely being sequestered in the BBB since the recovery from the MDCK assay was only 54%. The time course profile for the 30 mpk dose is analogous to Figure 4.

We sought to exploit the fast-in-slow-out profile of **16** by driving the brain levels higher via a BID dosing paradigm at 10 and 30 mpk. Gratifyingly, dosing **16** at 30 mpk BID over a period of 2.5 days led to a reduction in cortical and CSF Aβ of 28% and 32%, respectively (Fig. 5). Central Aβ was unaffected after administering **16** orally BID at 10 mpk for 2.5 days. The mean brain levels at 10 and 30 mpk following the final dose were 0.46 and 7.85 μM, respectively, with brain-to-plasma ratios of 0.63 and 0.71, respectively. This nonlinear correlation compelled us to reexamine the time course profile of the compound.

Inspection of the time course profile of **16** administered orally at 100 mpk revealed that the brain levels had flat lined, but the plasma levels also plateaued (Fig. 6). This phenomenon is indicative of saturating the clearance mechanism. Cyclopropylamines are known to be time-dependant CYP inhibitors (TDI),[34] and indeed

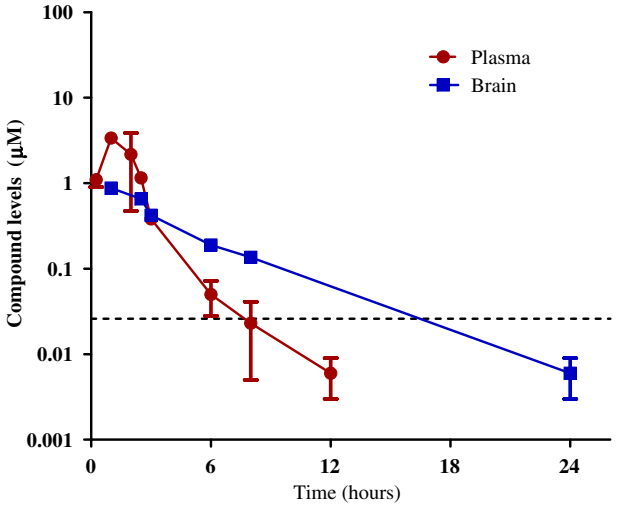


Figure 4. Twenty-four hour time course profile of the brain (blue) and plasma (red) levels of **16** in guinea pigs after dosing orally at 10 mpk. The dotted line is the cellular ED_{50}.

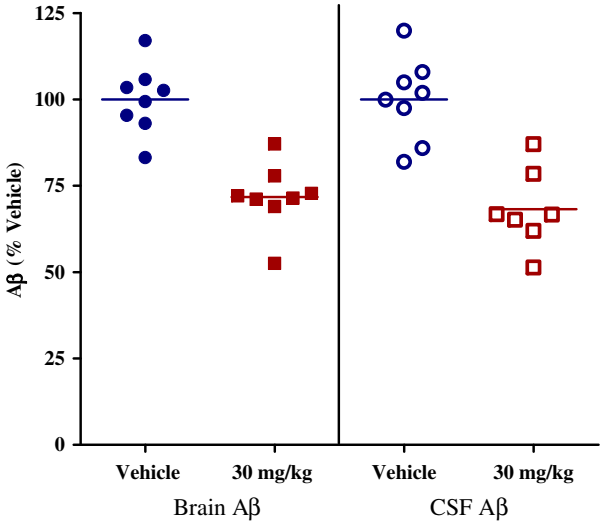


Figure 5. Reduction of cortical and CSF Aβ1-x by **16** in Hartley guinea pigs 6 h post final dose. Each circle or square represents one animal.[33]

16 proved to be culpable of this undesirable attribute. Preincubation **16** with and without NADPH led to an IC_{50} of 0.004 and 0.046 μM, respectively, giving an 11-fold shift against CYP3A4.[35] In addition to being a TDI, **16** is also a competitive inhibitor of CYP3A4 with an IC_{50} of 0.11 μM.

To further improve the potency and abrogate the time-dependent inhibition, we explored increasing the lipophilicity in the *P*1′. Cyclobutylamines undergo a similar P450 catalyzed ring opening[36] but do not inhibit the CYPs in a time-dependent fashion due to the slower rate of ring opening.[37]Unfortunately, the *P*1′ cyclobutyl moiety **18** lacked the desired potency. Additionally, this method suffers from limitations since even slight increases to the lipophilicity, **19**, are detrimental to both the permeability and recovery.

In conclusion, we have designed highly permeable HEAs with very low levels of P-gp liability and this translated into an orally efficacious inhibitor in the *wildtype* preclinical guinea pig animal model. The decisive element for success was to focus on engineering in the appropriate pharmacokinetic parameters, not the potency, of the inhibitor. This was accomplished by modulating

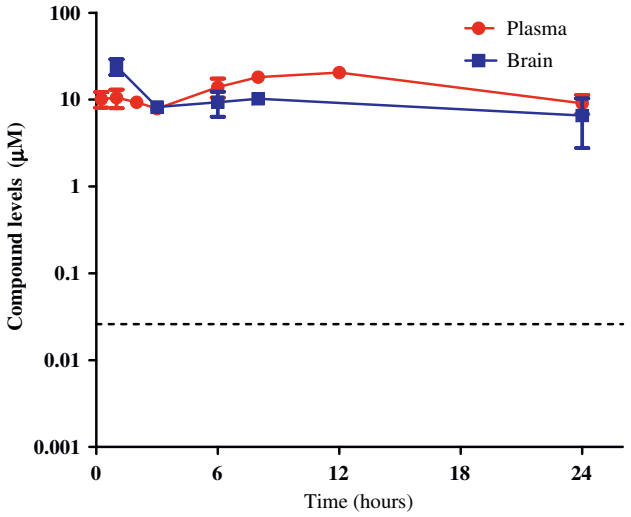


Figure 6. Twenty-four hour time course profile of the brain (blue) and plasma (red) levels of **16** in guinea pigs after dosing orally at 100 mpk. The dotted line is the cellular ED_{50}.

the pK_a of the amine with a cyclopropyl moiety, and generating polarity and/or lowering the log *P* on the *P2′* hydrocarbon with fluorine. The cellular activity may be a more pertinent measurement with which to judge an inhibitor, rather than enzymatic potency, since **16** is 26 nM in the cell and only 230 nM in the biochemical assay. The cell-to-enzyme ratios were excellent possibly due to compartmentalization,[38] which could result in higher local concentrations of the inhibitor relative to the BACE-1 enzymatic assay. Although this class of inhibitors had limitations, this research served as a springboard for future efforts.

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