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Optimization of a Natural Product-Based Class of γ-Secretase Modulators

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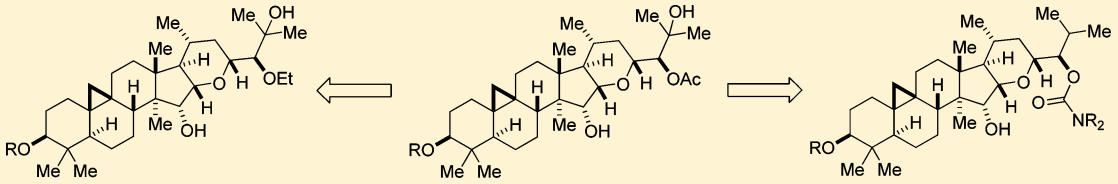
Supporting Information



ABSTRACT: A series of triterpene-based γ-secretase modulators is optimized. An acetate present at the C24 position of the natural product was replaced with either carbamates or ethers to provide compounds with better metabolic stability. With one of those pharmacophores in place at C24, morpholines or carbamates were installed at the C3 position to refine the physicochemical properties of the analogues. This strategy gave compounds with low clearance and good distribution into the central nervous system (CNS) of CD-1 mice. Two of these compounds, **100** and **120**, were tested for a pharmacodynamic effect in the strain and lowered brain Aβ42 levels.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and is characterized by impaired memory and cognition. It is estimated that 5.4 million people in the United States[1] and 35.6 million people worldwide[2] suffer from AD, and the number is expected to increase to 13.5 million[1] in the United States and 115 million[2] worldwide by 2050 because of the aging population. Moreover, AD prevalence will impact societies because of both the psychological burden to families and caregivers and the staggering economic costs incurred for long-term care of AD patients. The total cost paid for AD care in the United States was approximately $200 billion in 2012,[1] with the majority of that money coming from Medicare and Medicaid. By 2050, that cost is expected to rise to $1 trillion.[3] These statistics highlight the significant opportunity for social and economic impact that may be provided by an effective AD treatment.

A characteristic pathology found in Alzheimer's disease is the accumulation of amyloid-β (Aβ) peptides and the formation of plaques.[4] Amyloid-β peptides are formed when amyloid precursor protein (APP) is cleaved sequentially by the two membrane-bound aspartyl proteases, β-secretase and γ-secretase. This fact has inspired drug discovery scientists to develop both β-secretase and γ-secretase inhibitors as potential therapies that would prevent or slow AD development.[5] Inhibitor approaches are challenged by the issue that both β-secretase and γ-secretase cleavages are not specific to APP; in fact, γ-secretase cleaves more than 50 other substrates.[6] Of these, Notch cleavage by γ-secretase is biologically essential and GSI-based AD therapies result in the characteristic toxicities (including skin cancer and severe gastrointestinal effects[7]) that, in part, led to the phase III failure of Lilly's clinical candidate, Semagacestat.[8,9]

A closer look at the role Aβ plays in Alzheimer's disease reveals potential alternatives to β- and γ-secretase inhibition. Studies have shown that the total amount of Aβ produced in patients with familial Alzheimer's disease (FAD) is not necessarily higher than in normal individuals.[9] Therefore, inhibiting β- or γ-secretase might not be expected to be useful in treating the disease. Moreover, patients with FAD have mutations that occur either in presenilin, a γ-secretase enzyme component, or in amyloid precursor protein (APP), the polypeptide that is cleaved to form Aβ.[10] Some forms of FAD result from mutations in presenilin that cause γ-secretase to produce longer Aβ fragments such as Aβ42 and Aβ43 at the expense of shorter ones such as Aβ37−Aβ40. This fact suggests that a compound that could increase the ratio of shorter peptides relative to the longer ones without affecting the total amount of APP processed or Aβ produced may prove effective in treating AD. Significant effort by multiple groups has been devoted to finding such molecules and many have been described in patent applications and journal articles.[11] These molecules have been called γ-secretase modulators (GSMs), a name that helps distinguish them from GSIs, and points to the fact that they only modulate *where* cleavage occurs on APP and other substrates rather than inhibit all processing. Therefore, a critical benefit found in molecules that act through this mechanism is that they do not inhibit cleavage of Notch and

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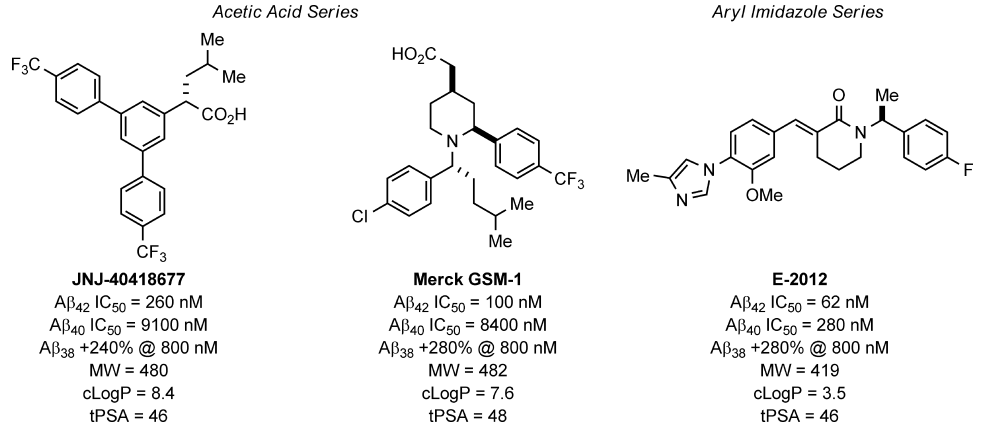

Figure 1. Classes of GSMs from the literature.

should therefore avoid the Notch-related toxicities seen with GSIs in the clinic.

In the dozens of literature reports on GSMs, the molecules can readily be placed in two general categories, the acetic acids exemplified by JNJ-4041877 and Merck GSM-1 and the aryl imidazoles exemplified by E-2012 (Figure 1). A noteworthy feature of nearly all these molecules[12] is that they increase Aβ38 levels at the expense of Aβ42. From a drug development perspective, one issue with the acid-containing GSMs is their very high lipophilicity. This could partly explain why GSMs, especially in this class, have made little progress in the clinic despite the significant resources that has been devoted to the discovery and development of a GSM-based AD therapy.

To identify novel GSMs, we screened known pharmaceutical agents and natural product extracts for their ability to lower Aβ42 relative to Aβ40 in a cell-based assay.[13] We found that out of more than 2000 samples, only one, black cohosh (*Actaea racemosa*) extract, accomplished this goal. Activity-guided fractionation afforded triterpene glycoside **1** as the most potent GSM in the extract, with an IC_{50} of 100 nM against Aβ42 and 6300 nM against Aβ40 (Figure 2).[14] This natural product also possessed an unusual profile relative to other GSMs in that it raised Aβ37 and Aβ39 while lowering Aβ38 and Aβ42.[14]

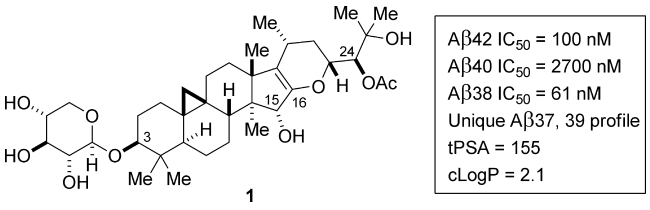

Figure 2. Triterpene glycoside **1** as an initial hit.

Although compound **1** possessed excellent potency against Aβ42 and an intriguing profile against the other Aβ-peptides, lowering Aβ38 while raising levels of Aβ37 and not affecting Aβ40, it also contained several structural liabilities that prevent it from being a viable drug for a central nervous system (CNS) disease. First, the enol ether at C16 is expected to cause chemical stability problems. In addition, the sugar makes a significant contribution to the high tPSA calculated for **1**, a property that has been strongly correlated with poor CNS permeability in the literature.[15] Third, the C24 acetate is expected to exhibit poor metabolic and chemical stability. The latter issue was confirmed in an early mouse pharmacokinetics experiment where we found that C24 deacetylation is the major metabolite following oral administration of **1**. The goal of our program is to address these issues to create a compound that retains the excellent pharmacological profile of natural product **1** while improving its chemical, metabolic, and distribution properties. This will allow the robust CNS exposure needed to safely elicit a robust pharmacodynamic response in animals and ultimately in people.

We recently reported our efforts to address the liabilities associated with the enol ether and the sugar (Scheme 1).[16,17] It was found that reducing the enol ether to give the *trans* fusion at the D–E ring juncture gives compound **2** with slightly improved (60 nM) potency at lowering Aβ42. We also found that replacing the sugar with carbamates[17] as in compound **3** (IC_{50} = 47 nM) or with morpholines[16] as in compound **4** (IC_{50} = 70 nM) gave analogues with good potency, reduced tPSA, and the ability to enter the CNS. These compounds, however, did not possess the metabolic stability sufficient to give therapeutically useful drug exposure levels.

This article describes our design and synthesis efforts to increase the overall metabolic stability of this triterpene GSM series through replacement of the acetate-containing C24–C27 side chain. We then describe our efforts to further refine the C3 alcohol substituent to obtain molecules that display sufficient CNS exposure to give a robust PD response in mice. To make progress toward this end, we filtered our compounds through an appropriate screening funnel, a simplified version of which is shown in Figure 3. In the first tier of screening, compounds were advanced based on for good potency at lowering Aβ42 and selectivity versus Aβ40.[18] Aβ40 was measured because it represents the vast majority of the pool of total Aβ and thus is an important peptide to follow to confirm selectivity. Also in the first tier of screening, stability in human liver microsomes was measured in the absence of NADPH. The assay data without NADPH present was used as a qualitative measure of the susceptibility of a particular compound to enzymatic hydrolysis. Compounds that met those in vitro criteria would be tested for their ability to maintain therapeutically relevant plasma levels and cross the blood–brain barrier in mice[19] and, in turn, compounds that passed that hurdle would be tested for their ability to elicit a PD response in mice, which we defined as greater than or equal to 25% lowering of Aβ42 levels.[20]

RESULTS AND DISCUSSION

As a first step toward building potent and more metabolically stable alternatives to the C24–C27 side chain present in natural

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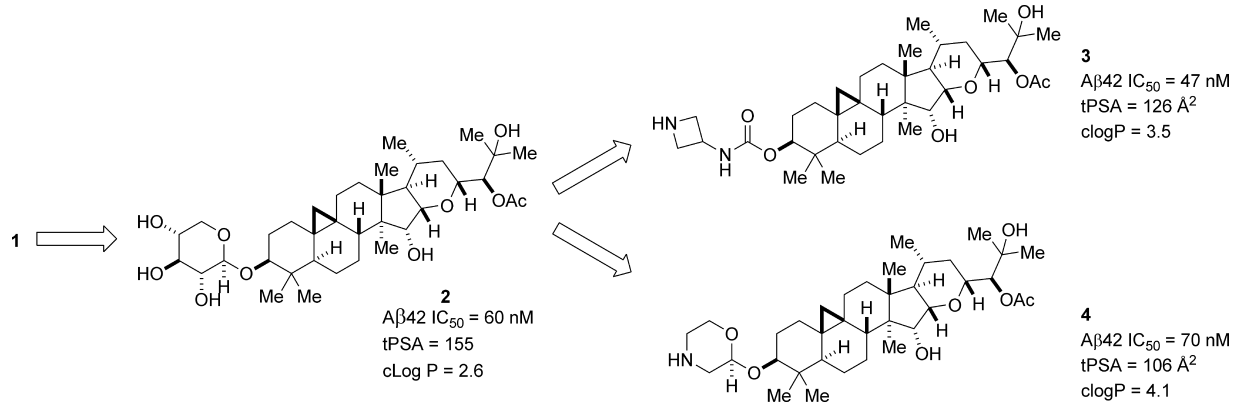
Scheme 1



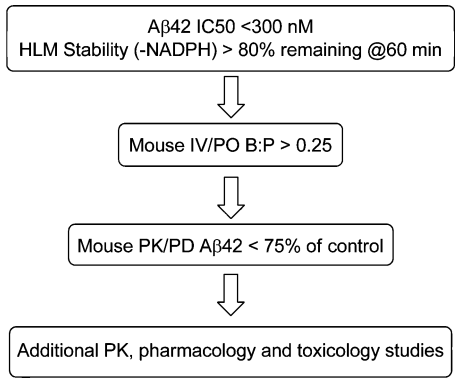
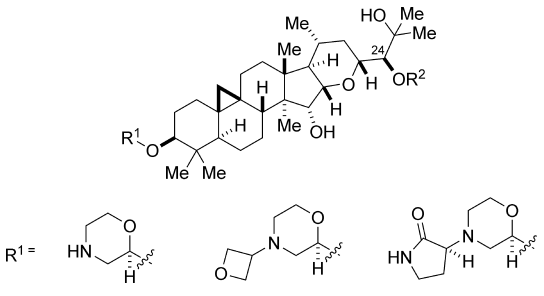


Figure 3. Simplified screening funnel.

Table 1. Comparison of C24 Acetates to C24 Alcohols

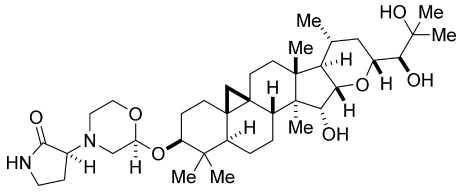


5: R^2 = Ac **8:** R^2 = H | **6:** R^2 = Ac **9:** R^2 = H | **7:** R^2 = Ac **10:** R^2 = H

compd	$A\beta_{42}$ C_{50} (nM)	HLM stability[a] (−NADPH)	HLM stability (+NADPH)
5	56	7	3
6	170	1	1
7	170	3	63
8	1600	112	40
9	≥20000	107	81
10	≥20000	85	

[a]Percent parent remaining at 60 min.

product **1**, we hydrolyzed the acetate in a series of three potent morpholines that otherwise possessed good properties (Table 1). These resulting compounds (**5**−**10**) were 30- (**8**) to >230-fold (**9**, **10**) less potent than the corresponding acetates; however, in the absence (and presence) of NADPH, they all possessed much better stability in human liver microsomes than the acetates.

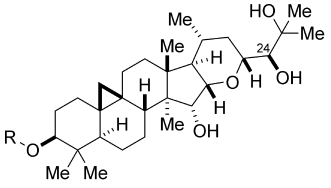


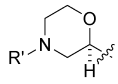
14

3 mpk IV	10 mpk PO
Cl = 0.58 L/hr/kg	Cmax = 2.2 μM
V_d = 3.10 L/kg	%F = >100%
$t_{1/2}$ = 5.0 h	B/P = 0.09 (6 h)

Figure 4. Rat PK for compound **10**.

Table 2. Aβ42 Lowering by C24 Alcohols with Various C3 Groups





M

C

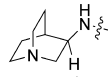
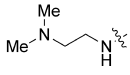
compd	R	R'	$A\beta_{42}$ IC_{50} with C24 OAc (nM)	$A\beta_{42}$ IC_{50} (nM)/ % reduction
11	M		70	40% @ 4 μM
12	M		70	50% @20 μM
13	M		60	0% @ 20 μM
14	M		130	5% @ 20 μM
15	C		340	13000
16	C		85	580
17	C		220	9000
18	C		360	5700

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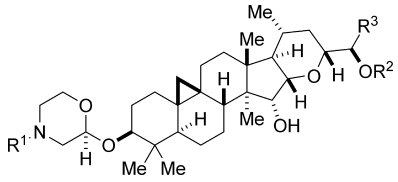
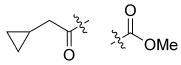
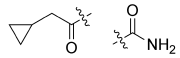
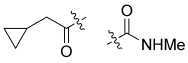
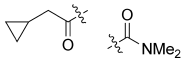
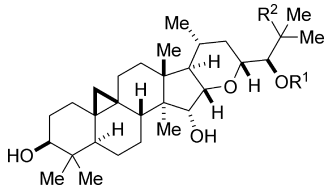
Table 3. Analogues without a C25−C27 Fragment



compd	R^1	R^2	R^3	$A\beta_{42}$ IC_{50} (nM)/ % reduction	$A\beta_{40}$ IC_{50} (nM)/ % reduction	HLM stability (-NADPH)[a]
19		OAc		170	35% @ 20 μM	1
20		OAc		130	38% @ 20 μM	2
21		OAc	H	0% @ 20 μM	0% @ 20 μM	0
22		OAc	H	240	66% @ 100 μM	23
23			H	330	46% @ 20 μM	59
24			H	840	35,000	77
25			H	350	81000	81
26			H	1200	63% @ 20 μM	81

[a]Percent parent remaining at 60 min.

Table 4. Initial C24 Carbamate and Ether Analogues

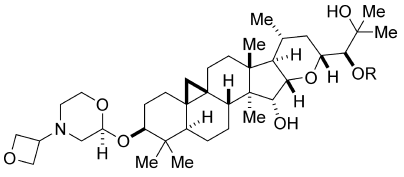


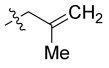
compd	R^1	R^2	$A\beta_{42}$ IC_{50} (nM)/ % reduction	$A\beta_{40}$ IC_{50} (nM)/ % reduction	HLM stability[a] (-NADPH)
27	Ac	OH	600	ND	ND
28	Ac	H	780	7500	0
29		OH	27% @ 20 μM	ND	96
30		H	890	8500	96
31		OH	53% @ 20 μM	ND	115
32		H	1000	10000	81
33	Et	OH	350	5500	100
34	Et	H	4400	ND	85

[a]Percent parent remained at 60 min.

des-Acetyl compound **10** was dosed in rats in an IV/PO pharmacokinetics experiment to test the hypothesis that removal of the metabolically labile ester would result in low clearance in vivo (Figure 4). We were encouraged to see that this compound gave low clearance and good oral bioavailability, indicating that the core scaffold of this series was not prone to significant metabolic liabilities. The concentration of **10** in the brain at 6 h was determined to provide a preliminary assessment of CNS penetration, and the resulting brain to plasma ratio of 0.09 indicated that this property would need to be refined.

Table 5. C24 Ether SAR



compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50} (nM)	HLM stability[a] (-NADPH)
35	Me	690	9900	93
36	Et	270	4600	94
37	*i*-Pr	440	2800	105
38		340	11000	84
39		3000	6000	78

[a]Percent parent remaining at 60 min.

The improved metabolic stability of the C24−C25 diol led us to examine whether a significant portion of potency to lower Aβ42 could be retained in the absence of the acetate by selecting an appropriate C3 group. This idea was tested by taking a diverse and potent set of C24 acetates and hydrolyzing the ester to produce the target compounds (Table 2). We found that the C3 carbamates generally gave the most potent C24−C25 diols with the basic carbamates **16** (IC_{50} = 580 nM) and **18** (IC_{50} = 5700 nM) being the most active. It seemed unlikely, however, that we would be able to obtain compounds with potencies lower than 300 nM using this strategy and this motivated us to seek alternatives.

Dissecting the C24−C27 fragment further, we decided to interrogate the role of the C25−C27 group through preparation of analogues with a C24 primary alcohol derivative. We gathered initial data to help answer this question (Table 3) by replacing the acetate-containing C24−C27 fragment in two potent analogues (**19**, IC_{50} = 170 nM, and **20**, IC_{50} = 130 nM) with the C24 primary alcohol-derived acetate. It was surprising to discover that, unlike in the series bearing the native C24 side chain, in the series lacking the C25−C27 fragment, the C3 group profoundly impacted the analogue potency with acyl morpholine **22** (IC_{50} = 240 nM) being dramatically more potent than oxetanyl morpholine **21** (IC_{50} > 20 μM). The potent Aβ42 lowering effected by **22** prompted us to examine acetate bioisosteres that might afford improved metabolic stability relative to **22** (compounds **23**−**26**). Of these, the methyl carbonate (**23**, IC_{50} = 330 nM) and the methyl carbamate (**25**, IC_{50} = 350 nM) also have good potency and improved metabolic stability. Additional SAR of these C24-alcohol analogues with C3 groups other than the acyl morpoline gave compounds with diminished potency and prompted us to design other replacements for the C24−C27 fragment.

We returned to examining bioisostere replacements with the C25−C27 fragment intact. When we looked at available bioisosteres for the metabolically labile ester bond, two

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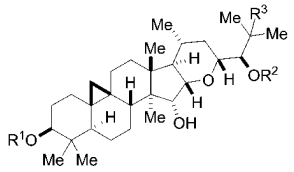
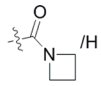
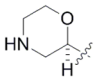
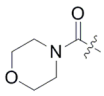
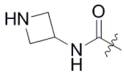
Table 6. Matrix of C24 Substituents with Varied C3 Groups



Legend: **Compound ID**
Aβ42 IC_{50} (nM)
Aβ40 IC_{50} (nM)
HLM % remaining at 60 min (-NADPH)

R^1 \ R^2/R^3	Ac/OH	OEt/OH	$CONH_2$/H	CONHMe/H	$CONMe_2$/H	
	**4** 60 nM 2200 nM 7%	**40** 91 nM 1400 nM 28%	**41** 650 nM 1700 nM 108%	**42** 480 nM 4300 nM 95%	**43** **580 nM** 5900 nM 114%	**44** 260 nM 7900 nM 123%
	**45** 200 nM 37% @ 20 μM ND	**46** 210 nM 3900 nM 116%	**47** 700 nM 1200 nM 103%	**48** 950 nM 40% @ 20 μM 117%	**49** 920 nM 19000 nM 131%	**50** ND ND 150%
	**51** 310 nM 14000 nM 2%	**52** 220 nM 2000 nM 77%	**53** **3400 nM** **32% @ 20 μM** **130%**	**54** **4600 nM** **17% @ 20 μM** **139%**	**55** **7400 nM** **17% @ 20 μM** **118%**	**56** 360 nM 11000 nM 104%
	**3** 47 nM 15000 nM 21%	**57** 53 nM 1900 nM 92%	**58** 160 nM 4000 nM 121%	**59** 120 nM 5000 nM 110%	**60** 300 nM 9500 nM 113%	**61** 100 nM 4800 nM 127%
	**62** 510 nM 15000 nM 17%	**63** 140 nM 5000 nM ND	**64** **40% @ 20 μM** **15% @ 20 μM** **106%**	**65** **17,000 nM** **47% @ 20 μM** **152%**	**66** **0% @ 20 μM** **0% @ 20 μM** **127%**	**67** 470 nM 10000 nM 158%

possibilities seemed to stand out: carbamates and ethers.[21] Carbamates were attractive because they contained a carbonyl group that might act as a necessary hydrogen-bond acceptor in interactions with the enzyme and, in addition, a diverse set of carbamates could be prepared by allowing the appropriate intermediate to react with different amines. At first glance, ethers seemed less attractive than carbamates because they did not possess the carbonyl group that conferred similarity to the esters but it was possible that they would fill the same enzymatic pocket as the ester or exert a conformational effect on the C25−C27 fragment that could be important for potency. In making an initial set of carbamates and ethers, we also wanted to explore the role that the C25 hydroxyl group played in the Aβ42 lowering potency of these compounds. As an added benefit, we knew that removing the C25 hydroxyl group would simplify the preparation of certain carbamate analogues because the C25 hydroxyl is capable of cyclizing onto the activated intermediate at C24.

The Aβ42 lowering potency and microsomal stability results for these compounds are shown in Table 4. A pattern that immediately appears is that the hydroxyl group can play an important role in the activity of the compounds depending on the identity of the C24 substituent. The C24 acetate with a hydroxyl group at C25 (**27**) has a similar IC_{50} (600 nM) for lowering Aβ42 as the compound without (**28**, IC_{50} = 780 nM). This SAR pattern is not maintained with the carbamates, where compounds without a hydroxyl group (**30**, IC_{50} = 890 nM and **32**, IC_{50} = 1000 nM) have better potency than those that possess one (**29**, 27% lowering at 20 μM, and **31**, 53% lowering at 20 μM). It is also very interesting that, compared to the carbamates, the ethers display the opposite pattern where the compound with a hydroxyl group (**33**, IC_{50} = 350 nM) is more potent than the one without (**34**, IC_{50} = 4400 nM). These results suggest that subtle conformational effects are having a significant impact on potency and provide further evidence that this region of the molecule participates in a critical interaction with γ-secretase. Important for our overall objectives, all of these compounds show good stability against hydrolytic enzymes present in microsomes.

On the basis of these compelling results, we wanted to examine how different C24 ethers would affect analogue potency with the oxetane morpholine at the C3 position (Table 5). We found that ethyl ether **36** (IC_{50} = 270 nM) gave the best potency, with compounds **35** (IC_{50} = 690 nM), **37** (IC_{50} = 440 nM), and **38** (IC_{50} = 340 nM) being within a 3-fold potency range. Isobutenyl ether **38** gave similar potency to compound **36**, but we chose to focus on the ethyl ether (**36**) in future analogues because it would impart a lower log *P* and does not contain a double bond, which could cause problems arising from metabolism and bioactivation. It is notable that compound **39** was much less potent (IC_{50} = 3000 nM) than the others, and this suggests that there is a size limit for this pharmacophore.

We were very encouraged by these preliminary results with the C24 carbamates and ethers and focused our attention on a more thorough interrogation of the C3 group in conjunction with the C24 substituent through the design and synthesis of a 5 × 6 matrix of analogues. For the C3 groups, we chose a morpholine and a combination of basic and neutral carbamates, and for the C24−C27 substituent, we examined C24 acetate or ethyl ether with a C25-hydroxyl group and four different C24 carbamates without a C25-hydroxyl group. The carbamates were chosen based on two criteria: simplicity and different degrees of substitution on nitrogen. To this end, the primary, methyl and dimethyl carbamates were made for each C3 group, along with the azetidine carbamates, with the idea that the four-membered ring would attenuate potential oxidative dealkylation. This matrix of analogues (Table 6) revealed useful

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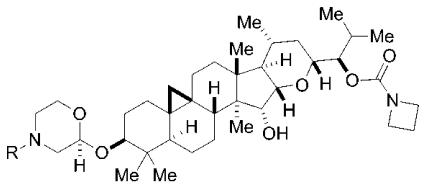
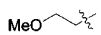
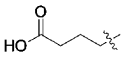
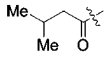
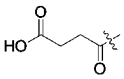
Table 7. SAR of C24 Carbamates with a C3 Morpholine



compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50} (nM)/ % reduction	Ionization[a] (cpKa)	clogP	tPSA
44	H	260	7900	B (7.7)	5.3	89
68	Me	770	8400	B (6.3)	5.7	81
69		530	5200	B (6.3)	5.7	90
70		560	11000	B (4.6)	5.6	90
71		490	15000	B (5.1)	4.7	110
72		700	41% @ 20 μM	Z (5.3, 3.2)	4.1	118
73		280	7500	Z (7.0, 3.8)	3.0	118
74		400	11000	N	6.2	98
75		1300	11000	N	6.4	98
76		280	7200	A (4.0)	4.8	135
77		110	7700	B (6.8)	4.7	101
78		1300	49% @ 20 μM	A (10.0)	5.4	144

[a]B = basic (pK_a for the conjugate acid is shown), A = acidic, Z = zwitterionic, N = neutral

trends, and in the table compounds with an IC_{50} above 1 μM are illustrated in red. Three C3 groups were found that give acceptable potency across the range of C24 substituents, including the morpholine (compounds **4, 40**−**77**), the primary carbamates (**45**−**50**), and the amino azetidine carbamate (compounds **3**, **57**−**61**). A similar analysis carried out on the C24 isosteres revealed that the ethers (**40**, **46**, **52**, **57**, **63**), and azetidine carbamates (**44**, **50**, **56**, **61**, **67**) give IC_{50} values of less than 1 μM across the full set of C3 groups. On the basis of these results, the C24 ethers and C24 azetidine carbamates were chosen for further optimization.

Analogues that possessed a C24 ethyl ether or azetidine carbamate were optimized by installing a variety of C3 substituents that we believed could give our compounds the right combination of attributes to progress through downstream assays.[15] We especially wanted to build ionizable centers with varied pK_as into our molecules as we knew that these would dramatically affect the pharmacokinetics of our compounds and our ability to develop a suitable drug product. Basic compounds were particularly interesting because we believed that they would give us highest probability for CNS penetration. In designing these compounds, we wanted a significant portion to have calculated pK_a values of less than 8 because lipophilic basic amines have been associated with toxicity.[15] Other design criteria included keeping the clogP and tPSA as low as possible to maximize the probability that these compounds would penetrate the CNS. In addition, high clogP has been strongly associated with off-target pharmacology and idiosyncratic toxicity in the literature.[15] Because of this, we also decided to include some carboxylic acids in our analogues, which would have a significant effect on lowering logP, even though we knew that these compounds were less likely to enter the CNS. Because of the molecular weight of our scaffold, our ability to lower tPSA and clogP to a range more typically seen in a CNS compound was limited, but we wanted to maintain these principles in analogue design. We also realized that these guidelines were developed primarily using molecules very different from our scaffold. Concerns about the inherent physicochemical properties of our scaffold were mitigated by analyses indicating that natural product derived therapeutics require less stringent adherence to these guidelines.[22]

The first set of analogues we designed for in-depth examination was the C24 carbamates with a C3 morpholine (Table 7). This set of compounds contained six basic compounds with calculated pK_a values between 4.6 and 7.7 and two each of neutral compounds, acidic compounds, and zwitterionic compounds. There was no general correlation between ionization class at C3 and potency, indicating that enzyme binding to this region of our scaffold tolerates each of these compound types. With the exception of triflamide **78** (IC_{50} = 1300 nM), all of the analogues prepared in the set had an IC_{50} of less than 1 μM but generally did not hit our 300 nM goal and were less potent than the corresponding C24 acetates.[15] The most potent compounds were unsubstituted morpholine **44** (IC_{50} = 260 nM), zwitterionic compound **73** (IC_{50} = 280 nM), acid **76** (IC_{50} = 280 nM), and amine **77** (IC_{50} = 110 nM). This relatively small set of compounds reveals no distinct SAR patterns with the possible exception that compounds with an *N*-acyl link to an ionized functional group gave good potency (compounds **76**, IC_{50} = 280 nM, and **77**, IC_{50} = 110 nM). The carbamate acyl linkage proved stable in all of these examples as measured by human liver microsomal stability without NADPH.

The next set of analogues possessed the azetidine carbamate at C24 and varied carbamates at C3 (Table 8). This set contained five basic compounds with calculated pK_a values between 3.9 and 9.1, 12 neutral compounds, and five acidic compounds. In this set of compounds, those with an amine that had a pK_a of greater than 6 all had good potency (**81**, IC_{50} = 100 nM; **61**, IC_{50} = 170 nM; **90**, IC_{50} = 91 nM; **92**, IC_{50} = 170 nM). The compounds that contained a piperazine ring (**92**, IC_{50} = 170 nM, and **93**, 190 nM) also provided good $A\beta42$ lowering capabilities irrespective of ionization state, suggesting that there is a feature within the six-membered piperazine ring other than physicochemical properties that promotes potent $A\beta42$ lowering. One out of five acids met our pharmacology criteria (**89**, IC_{50} = 280 nM), and among the 12 neutral compounds, two of the carbamates (**95** and **96**) had an IC_{50} of less than 300 nM, each of which contained a highly polar lactam ring. As was the case with the compounds in Table 7, these analogues were all stable to enzymatic hydrolysis in human liver microsomes. On the basis of our combined results, we were confident that replacing the C24 acetate with a carbamate on an appropriate C24−C27 fragment would shut down this metabolic liability despite the theoretical possibility

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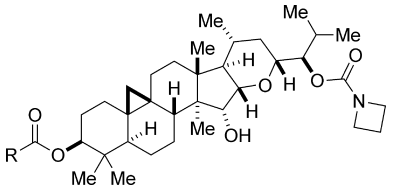
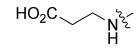
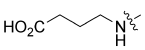
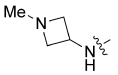
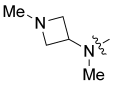
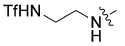
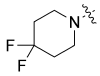
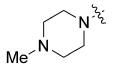
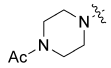
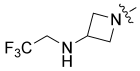
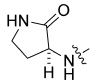
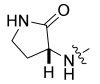
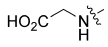
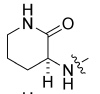
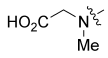
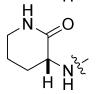
Table 8. SAR of C24 Carbamates with a C3 Carbamate



compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50}(nM)/ % reduction	Ionization[a] (pKa)	clogP	tPSA	compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50} (nM)	Ionization[a] (cpKa)	clogP	tPSA
79		760	8700	N	5.3	97	**88**		610	13000	A (4.3)	5.0	135
80		1300	1400	N	5.5	89	**89**		280	7200	A (3.9)	5.3	135
67		470	10000	N	5.4	89	**61**		170	6500	B (6.5)	5.2	101
81		100	2600	B (9.1)	4.8	109	**90**		91	5400	B (6.6)	5.4	92
82		350	8000	N	4.7	109	**91**		1500	11000	A (6.1)	5.9	144
83		9100	12% @ 20 μM	N	6.6	89	**92**		170	8200	B (8.4)	5.4	92
56		360	11000	N	5.3	9.8	**93**		190	5100	N	4.6	109
84		750	11000	B (3.9)	6.0	101	**94**		400	1000	N	4.3	126
85		3000	27% @ 20 μM	N	5.2	106	**95**		180	5900	N	4.3	126
86		420	7200	A (3.8)	4.8	135	**96**		280	6400	N	4.8	126
87		1793	480	A (3.9)	5.0	126	**97**		380	6600	N	4.8	126

[a]B = basic (pK_a for the conjugate acid is shown), A = acidic, Z = zwitterionic, N = neutral

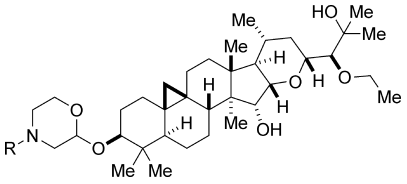
that this functional group could also be enzymatically hydrolyzed.

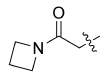
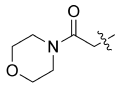
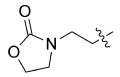
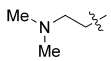
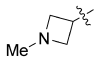
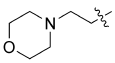
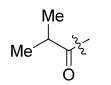
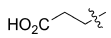
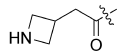
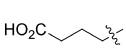
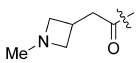
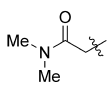
With a diverse set of several potent C24 carbamates in hand for further testing, we returned our attention to C24 ethers. The first set of analogues we prepared were C3 morpholines (Table 9), and this group contained 24 basic compounds with calculated pK_as ranging from 0.7 to 9.5, two zwitterionic compounds, and one neutral compound. We were very pleased to find that of these 27 compounds, 19 met our 300 nM Aβ42 lowering potency cut off and all but three analogues (**103**, IC_{50} = 540 nM; **104** and **109**) had an IC_{50} of less than 500 nM. Out of the six of the compounds where a direct comparison was available, five were approximately 2- to 3-fold more potent than the corresponding C24 carbamates (see Table 7). Examples of such comparisons include **41** (IC_{50} = 91 nM) versus **44** (IC_{50} = 260 nM) and **100** (IC_{50} = 270 nM) versus **69** (530 nM). Because the majority of compounds show similarly good potency, the examples that do not show desired potency levels deserve comment. The trend in going from the potent monofluoro-compound **102** (IC_{50} = 240 nM) to less potent di- and trifluoro- compounds (**103**, IC_{50} = 540 nM; **104**, IC_{50} = 2300 nM) transforms a relatively polar and moderately basic functional group into one that is apolar and essentially neutral. This is consistent with the previously observed trend with C24 acetates that suggests that small (e.g., methyl or ethyl) or hydrophilic groups are preferred on the morpholine unless linked through and acyl group as in compound **121**.[16] The other compound that had an IC_{50} of more than 500 nM was carboxylic acid **109** (630 nM). It is noteworthy that C24 carbamate **73**, which contains the same acidic C3 morpholine as **109**, was the second most potent compound in the series with an IC_{50} of 280 nM (Table 7). This example demonstrates that although the pharmacological effects exerted by the C24–C27 fragment and C3 substituent are usually independent, this is not always the case.

A final set of compounds that was prepared possessed a C24 ethyl ether and a range of carbamates at C3 (Table 10). These compounds included eight basic analogues with pK_as ranging from 2.9 to 9.1, seven neutral analogues, three acidic analogues, and one zwitterionic analogue. The compounds were on

dx.doi.org/10.1021/jm300976b | J. Med. Chem. 2012, 55, 9270–9282

Table 9. SAR of C24 Ethers with a C3 Carbamate



compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50} (nM)/ % reduction	Ionization (pKa)	clogP	tPSA	compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50} (nM)/ % reduction	Ionization (cpKa)	clogP	tPSA
41	H	91	1400	B (7.7)	4.7	89	**111**		250	39% @ 4 mM	B (5.2)	4.0	101
98	Me	250	4500	B (6.3)	5.0	81	**112**		230	14000	B (5.2)	3.9	101
99	Et	210	2700	B (6.5)	5.4	81	**113**		150	1900	B (5.8)	4.7	110
100		270	9900	B (6.3)	5.0	90	**114**		180	3000	B (6.1)	4.9	90
101		260	3700	B (6.1)	4.4	101	**115**		420	6200	B (6.1)	4.9	90
102		240	4700	B (5.1)	5.2	81	**116**		350	5000	B (7.1)	5.0	90
103		540	37% @ 4 mM	B (3.4)	5.5	81	**117**		270	4600	B (4.6)	4.9	90
104		2300	44% @ 20 mM	B (0.7)	6.0	81	**118**		400	5800	B (4.9)	5.2	90
105		130	3200	B (8.3)	5.1	84	**119**		100	2400	B (9.5)	4.6	93
106		110	2900	B (6.8)	4.9	84	**120**		100	2100	B (7.4)	4.9	84
107		430	1300	B (7.1)	4.8	93	**121**		170	3500	N	5.5	98
108		310	9600	Z (6.8, 3.2)	2.6	118	**122**		120	2500	B (10.7)	3.8	110
109		630	6500	Z (7.0, 3.8)	2.3	118	**123**		150	3100	B (4.6)	4.2	101
110		270	38% @ 4 mM	B (5.2)	4.2	101							

[a]B = basic (pK_a for the conjugate acid is shown), A = acidic, Z = zwitterionic, N = neutral

average 2-fold more potent than the 10 directly comparable compounds with a C24 carbamate (Table 8). This potency difference ranged from between 1.3-fold (**135** IC_{50} = 140 nM vs **95** IC_{50} = 180 nM) to 3.4-fold (**63** IC_{50} = 140 nM vs **67** IC_{50} = 470 nM). We concluded on the basis of these data, in conjunction with the comparison between compounds in Tables 9 and 7, that the C24 ethyl ether is a more effective pharmacophore than the C24 azetidine cabamate. Of these 18 analogues, 13 met our Aβ42 reduction target and all of the compounds had IC_{50}s of less than 1 μM. In addition, several of the compounds in this set that contained a basic amine were among the most potent that had been prepared in the program. For instance, **57**, **126**, **127**, and **131** all had IC_{50} values of less than 100 nM for lowering levels of Aβ42, showing that this combination of C24 ethyl ether and C3 carbamate was a particularly potent. In this set of compounds, the acids were the least potent modulators of γ-secretase.

We were pleased to see such good potency across the large and physicochemically diverse set of compounds listed in Tables 7–10, and these compounds continued to maintain acceptable levels of metabolic stability in vitro (data not shown). We were confident that some would provide the pharmacokinetic properties necessary to elicit a pharmacodynamic response in animal models. To determine which of these compounds showed the most promise, several were screened for their ability to cross the blood–brain barrier. CD-1 mice were administered compound via IV route to measure the plasma concentrations and the brain-to-plasma ratio of our

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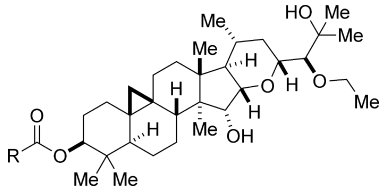
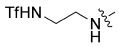
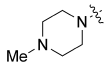
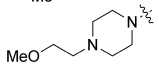
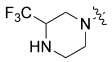
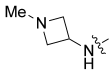
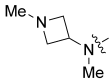
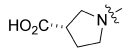
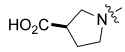
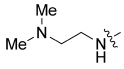
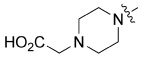
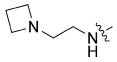
Table 10. SAR of C24 Ethers with a C3 Carbamate



compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50} (nM)/ % reduction	Ionization (pKa)	clogP	tPSA	compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50} (nM)/ % reduction	Ionization (pKa)	clogP	tPSA
46		210	3900	N	4.4	111	**130**		710	13% @ 20 μM	A (6.1)	5.2	143
124		360	47% @ 20 μM	N	4.6	97	**131**		68	5000	B (8.4)	4.7	92
125		140	5000	N	4.4	106	**132**		320	51% @ 20 μM	B (6.8)	4.6	101
57		53	1900	B (9.1)	4.1	109	**133**		390	31% @ 20 μM	B (2.9)	5.3	100
126		65	980	B (6.5)	4.5	100	**134**		190	2900	N	3.6	126
127		61	4300	B (6.6)	4.7	92	**135**		140	2500	N	3.6	126
63		140	5000	N	4.7	88	**136**		400	17000	A (4.3)	4.6	126
52		220	2000	N	4.6	98	**137**		540	14000	A (4.3)	4.6	126
128		240	17000	B (8.5)	4.6	100	**138**		290	10000	Z (7.5, 1.1)	1.6	129
129		220	41% @ 4 μM	B (7.5)	4.3	100							

[a]B = basic (pK_a for the conjugate acid is shown), A = acidic, Z = zwitterionic, N = neutral

compounds at 6 h post dose (with the exception of compound **44**, measured 2 h post dose). Several compounds were tested using this method, and the examples shown in Tables 11 and 12 best illustrate the factors that affect the ability of our compounds to cross into the CNS. The brain and plasma concentrations obtained were low relative to our expected therapeutic drug levels; however, we expected that these experiments would allow us to rank order our compounds with respect to this pharmacokinetic parameter. Other experiments not shown indicated that a basic nitrogen significantly enhanced CNS penetration, consistent with our expectations. As such, all the examples here contain this component. The experiments listed in Table 11 illustrate that C24 ethers are superior to C24 carbamates at crossing the blood–brain barrier. Across three pairs of compounds with identical C3 groups (**40** *B*:*P* = 1.67 and **44** *B*:*P* = 0.09,[23] **131** *B*:*P* = 0.13 and **92** *B*:*P* = 0.03, **127** *B*:*P* = 0.10 and **90** *B*:*P* = 0.04), the ether showed a 2.5- to 18-fold higher brain-to-plasma ratio. These data also suggested that C3 morpholines give better CNS levels than C3 carbamates when compounds **40** and **44** are compared to **131**, **92**, **127**, and **90**.

Table 12 shows several additional C24 ethyl ethers, and when combined with a C3 morpholine, these compounds consistently gave acceptable compound levels in the brain. These morpholines cover a range of basicities and include a diamine (**120**). The results for C3 carbamate **132** are given as a comparison to morpholine **100**, and the closely related structures provide further evidence that the carbamate linker present in **132** is associated with diminished CNS penetration. On the basis of these studies (Tables 10–11), we concluded the presence of a carbamate at C3 and/or C24 is associated with diminished CNS exposure of our compounds. The presence of this substructure seems to play a more important role than differences in physicochemical properties. For instance, from these tables, compounds **40** (clogP = 4.7, tPSA = 89), **98** (clogP = 5.0, tPSA = 81), **117** (clogP = 4.9, tPSA = 92), **100** (clogP = 5.0, tPSA = 90), and **120** (clogP = 4.9, tPSA = 84) without carbamates all give acceptable CNS penetration, whereas compounds **44** (clogP = 5.3, tPSA = 89), **131** (clogP = 4.7, tPSA = 92), **92** (clogP = 5.4, tPSA = 92), and **127** (clogP = 4.7, tPSA = 92) that possess carbamates show diminished ability to enter the CNS.

To bolster our hypothesis that the compounds with poor brain-to-plasma ratios were substrates for p-glycoprotein (P-gp), we elected to perform an experiment in MDR1 knockout mice that do not express P-gp. We chose bis-carbamate **90** as the test substrate and saw a brain-to-plasma ratio of 3.1 at 6 h after IP dosing at 10 mg/kg in these mice. When this compound was dosed in an identical manner in wild type FVB mice, the brain-to-plasma ratio was 0.035, which is an approximately 87-fold decrease. This experiment provided strong evidence that P-gp was responsible for the low brain exposure of that compound and other carbamates-containing analogues.

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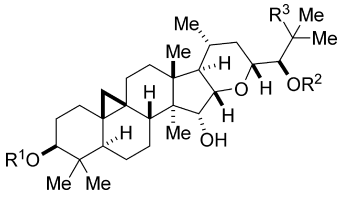
Table 11. Mouse (CD-1) IV Brain-to-Plasma Assessment for C24 Ethers and Carbamates



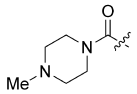
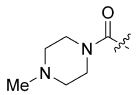
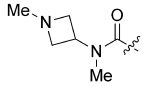
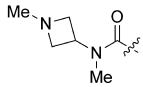
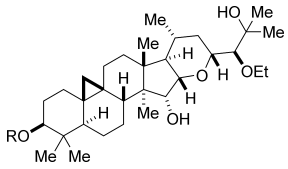
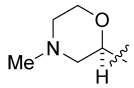
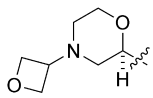
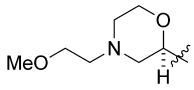
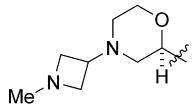
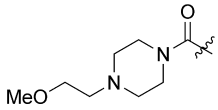
compd	R^1	R^2	R^3	Study	[Plasma]	[Brain]	B:P
40		Et	OH	3 mpk IV 6 h	192 nM	320 nM	1.67
44			H	1 mpk IV 2 h	326 nM	30 nM	0.09
131		Et	OH	1 mpk IV 6 h	95 nM	12 nM	0.13
92			H	1 mpk IV 6 h	450 nM	14 nM	0.03
127		Et	OH	1 mpk IV 6 h	566 nM	56 nM	0.10
90			H	1 mpk IV 6 h	274 nM	11 nM	0.04

Table 12. Mouse (CD-1) IV Brain-to-Plasma Assessment for C24 Ethers with C3 Ethers Carbamates



cmpd	R	Study	[Plasma]	[Brain]	B:P
98		1 mpk IV 6 h	21 nM	16 nM	0.76
117		3 mpk IV 6 h	24 nM	12 nM	0.50
100		1 mpk IV 6 h	35 nM	9 nM	0.26
120		1 mpk IV 6 h	107 nM	52 nM	0.49
132		1 mpk IV 6 h	261 nM	8 nM	0.03

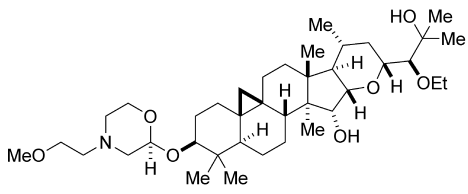
Compounds **100** and **120** were chosen for further evaluation, and their IV/PO pharmacokinetic profile in mouse is shown in Figure 5. Compound **100** gives low clearance (0.29 L/h/kg), low–moderate volume of distribution (0.42 L/kg), and relatively low bioavailability (19%). Compound **120** also gives lower clearance (0.15 L/h/kg), a higher volume of distribution (5.3 L/kg), and better bioavailability (75%). Both compounds give brain-to-plasma ratios that are consistent with the IV screening results (Tables 11 and 12). Plasma levels were sufficient to test the compounds in PK/PD experiments. These results also exemplify how we are able to attenuate certain properties, in this case V_d, by adjusting the overall physicochemical properties of the molecule by our choice of morpholine component.

A more comprehensive in vitro pharmacology profile for **100** and **120** is shown in Table 13. These two compounds lower Aβ38 with a similar IC_{50} to that observed with Aβ42, which is consistent with the unique GSM profile observed with our initial natural product hit (**1**). There was 37-fold ratio between Aβ42 and Aβ40 lowering for compound **100** and a 21-fold window for compound **120**, consistent with these compounds being GSMs. We also found, as expected, that these compound had no effect on total Aβ or Notch processing at concentrations where the compounds were not affecting cell viability.[24]

To better understand how our compounds preserved total Aβ, we quantified Aβ peptides in cells treated with compounds **100** and **120** using immunoprecipitation followed by LC/MS/MS (Table 14). The results for Aβ38 and Aβ42 were consistent with the results of our ELISA assay, where Aβ38 and Aβ42 were lowered. Compound **100** shows 58% Aβ38 lowering at 3 μM and Aβ42 is lowered below the limit of quantification. Compound **120** gives 33% lowering of Aβ38 at 2 μM and 83% lowering of Aβ42. Most significantly, both compounds raise Aβ37 and Aβ39. Compound **100** raised Ab37 and Aβ39 by 50% and 310%, respectively, and compound **120** raised Aβ37 and Aβ39 by 380% and 530%, respectively. These data, where Aβ37 and Aβ39 are raised while Aβ38 and Aβ42 are lowered, are consistent with the profile observed with compound **1**.[14]

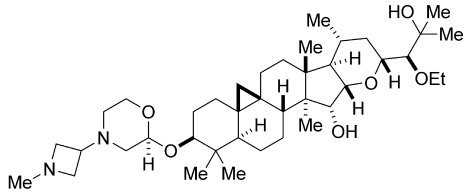
Given their good pharmacokinetic profile and their ability to effect a desirable in vitro pharmacological response, compounds **100** and **120** were taken into our CD-1 mouse PD assay. The CD-1 mouse was used because, in contrast to the transgenic overexpressing mouse models, the native level of expression may more accurately model the human condition of sporadic onset AD. Compounds **100** and **120** were dosed IP or PO once a day for either one or five days in CD-1 mice (Table 15). In the single-dose experiment, compound **100** showed statistically significant lowering of Aβ42, Aβ40, and Aβ38 at 100 mg/kg, with the strongest effect on Aβ42.[25] At this dose, a concentration of 12 μM in the plasma and 23 μM in the brain was achieved, and Aβ42 was lowered by 46%, Aβ40 by 30%, and Aβ38 by 31%. In a single-dose experiment, **120** resulted in a statistically significant lowering of Aβ42 and Aβ38 at the 30 and 100 mg/kg doses. This compound gave concentrations of 7.6 μM in the plasma and 5.6 μM in the brain at the 30 mg/kg dose, and these concentrations gave 16% lowering of both Aβ42 and Aβ38. At the higher 100 mg/kg dose, concentrations of 15 and 20 μM were observed in the plasma and brain, respectively, resulting in 26% lowering of Aβ42 and 31% lowering of Aβ38. In a 5-day study, statistically significant lowering was observed for **120** at 50 mg/kg, which gave concentrations of 16 μM in the plasma and 31 μM in the brain. These levels lowered Aβ42 by 42% and Aβ38 by 38%. Consistent Aβ42 and Aβ38 lowering across the experiments reflect the pharmacological effect observed in vitro. In all of these experiments, the amount of drug necessary to effect a

dx.doi.org/10.1021/jm300976b | J. Med. Chem. 2012, 55, 9270–9282



100

1 mpk IV	30 mpk PO
Cl = 0.29 L/hr/kg	Cmax = 3.6 μM
V_d = 0.42 L/kg	%F = 19
$t_{1/2}$ = 2.5 h	B/P = 0.40 (6 h)



120

1 mpk IV	30 mpk PO
Cl = 0.15 L/hr/kg	Cmax = 8.7 μM
V_d = 5.3 L/kg	%F = 75
$t_{1/2}$ = 39 h	B/P = 0.55 (6 h)

Figure 5. Mouse (CD-1) IV/PO PK profile for **100** and **120**.

Table 13. In Vitro Pharmacological Profile for 100 and 120

compd	Aβ42 IC_{50} (nM)	Aβ140 IC_{50} (nM)	Aβ38 IC_{50} (nM)	total Aβ IC_{50} (nM)	notch % lowering @ 4 μM	cell viability effect @ conc (μM)
100	270	9900	380	12000	0	20
120	100	2100	160	14000	0	20

Table 14. LC/MS/MS Quantification of Aβ Peptides

Aβ	1−37[3]	1−38[3]	1−39[a]	1−40[a]	1−42[a]
vehicle	0.6	1.2	1.0	10.2	0.6
100[b]	**0.9**	0.5	**3.1**	6.2	BLQ
120[c]	**2.3**	0.8	**5.3**	11.4	0.1

[a]Given in ng. Increases over vehicle are shown in bold. [b]Dosed at 3 μM. [c]Dosed at 2 μM.

pharmacodynamic response was significantly greater than the in vitro Aβ42 IC_{50} for **100** ([brain] = 20 μM, IC_{50} = 270 nM) and **120** ([brain] = 5.6, 20, 31 μM; IC_{50} = 100 nM). This might be explained by the high degree of plasma protein and brain homogenate binding observed with these compounds. The plasma and brain percent bound for **100** was 98.9% and 99.9%, respectively, and for **120** was 99.2% and 99.8%, respectively.[26]

CONCLUSION

We developed a series of potent GSMs that also possess a good pharmacokinetic profile and elicit a pharmacodynamic effect in vivo. Achieving the enhanced metabolic stability that translated into these desired in vivo properties required that the C24 acetate be replaced with more stable functionality. We initially tried to simplify the side chain that contained this acetate and install bioisosteres that might mimic the role the acetate plays in binding to γ-secretase. Although some of these compounds showed promising potency, the structure−activity relationships limited our ability to make a large and diverse set of potent compounds that would allow us to find candidates that could pass the hurdles downstream in our screening funnel. An in-depth examination of analogues that had a C24−C27 side chain similar to the natural product led us to find that C24 carbamates without a C25 hydroxyl group and C24 ethers with a C25 hydroxyl group afforded good potency and stability against enzymatic hydrolysis. We further found that these side chains allowed us to install a diverse set of substituents at C3 that could be used to impart a wide variety of physicochemical properties on the molecules. When a set of these was screened in vivo for their ability to enter the CNS, we found that a subset accomplished this task particularly well. Two representatives of this class, **100** and **120**, were then tested in PK/PD experiments and lowered Aβ42 in vivo. Further evaluation of these compounds and additional similar analogues continues, and results will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

Synthesis and analytical data for intermediates and exemplary compounds from each series. Details for the Aβ, microsomal stability, and notch assays and the rodent pharmacokinetics studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 15. IP or PO Mouse (CD-1) PK/PD Data for 100 and 120

	100	**100**	**120**	**120**	**120**	**120**
dose (mpk), route	30, IP	100, IP	30, PO	100. PO	10, PO	50, PO
paradigm	single dose	single dose	single dose	single dose	5-day multidose[c]	5-day multidose[c]
time point (h)	6	6	6	6	8	8
[plasma][a] (μM)	2.2	12	7.6	15	7.1	16
[brain][a] (μM)	1.6	23	5.6	20	7.0	31
Aβ42 lowering[b] (%)	7 ± 9	**46 ± 5**	**16 ± 12**	**26 + 11**	9 ± 15	**42 + 13**
Aβ40 lowering[b] (%)	8 ± 7	**30 ± 4**	9 ± 10	15 ± 10	+7 ± 10	10 ± 12
Aβ38 lowering[b] (%)	4 + 11	**31 ± 7**	**16 ± 13**	**31 ± 9**	13 + 9	**38 ± 10**

[a]μM. [b]Percent vehicle, brain homogenate. [c]Drug concentration and Aβ were measured on the last day of dosing **Bold** indicates statistically significant lowering: the number of animals used in the study was $N = 12$ per group. The value reported is mean ± the standard deviation. Using an ANOVA with a Dunnett's test, the p-value is <0.01. The same p value is achieved if a Tukey test is used as the posthoc test.

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All authors have given final approval to the final version of this manuscript.

Notes

The authors declare the following competing financial interest(s): The authors note the following relevant financial interests: the authors are named as inventors on one or more patents and patent applications related to compounds discussed in this paper and either hold equity and/or options on equity in Satori Pharmaceuticals.

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■ ABBREVIATIONS USED

Aβ, amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein; cpK_a, calculated inverse log of the acidity constant; cLogP, calculated log of the partition coefficient; CNS, central nervous system; FAD, familial Alzheimer's disease; GSM, γ-secretase modulator; GSI, γ-secretase inhibitor; HBD, hydrogen bond donors; IV, intravenous; mg/kg, milligrams per kilogram of body weight; PD, pharmacodynamic; P-gp, p-glycoprotein; PK, pharmacokinetic; PO, per os (by mouth); tPSA, topological polar surface area; SAR, structure−activity relationship

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(18) Defined as little or no lowering of Aβ40 at concentrations where Aβ42 is significantly lowered.

(19) A *B*:*P* ratio of 0.25 was chosen as our threshold because compounds with this degree of CNS penetration could provide sufficient brain concentrations to effect a PD response and may be superior to compounds with higher *B*:*P* ratios in other respects.

(20) This minimum criterion was derived from literature reports that demonstrate that cells expressing mutant forms of APP and PS1 that cause Alzheimer's disease have as little as a 25% increase in Aβ42. Page, R. M.; Bauman, K.; Tomioka, M.; Pérez-Revuelta, B. I.; Kukumori, A.; Jacobsen, H.; Flohr, A.; Luebbers, T.; Ozmen, L.; Steiner, H.; Haass, C. *J. Biol. Chem.* **2008**, *282*, 677−683.

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(23) Plasma and brain exposures for compound **44** were measured at an earlier time point as we were refining our assay protocol. We believe the *B*:*P* ratio is comparable to the other compounds because the IV dosing distribution phase is complete at 2 h.

(24) Compounds were determined to have a significant effect on cell viability when a >20% lowering of Cell Titer Glo (Promega) signal was observed. A >20% reduction was the minimum that was considered significant. Any significant effect on cell viability will also affect Aβ and Notch processing.

(25) Statistically significant Aβ40 lowering for **100** at 100 mg/kg is surprising given the difference between the in vitro Aβ42 IC_{50} (270 nM) and the Aβ40 IC_{50} (9900 nM); however, the in vivo data reported in Table 13 for compound **100** are collected at the single

time point after a single dose of compound and may not allow one to fully observe the selectivity of the compound. That being said our in vivo assay is capable of distinguishing a difference of 15% using a $N = 8$, indicating that the difference between lowering Aβ42 and Aβ40 is real and not due to variability. In addition, an ANOVA analysis indicates that the difference between Aβ40 and Aβ42 lowering is statistically significant, the p-value is <0.01.

(26) Measured by ultracentrifugation.