ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

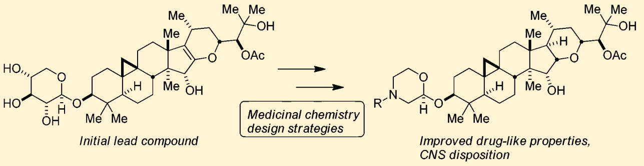
Initial Optimization of a New Series of γ-Secretase Modulators Derived from a Triterpene Glycoside

Nathan O. Fuller,* Jed L. Hubbs, Wesley F. Austin, Steffen P. Creaser,[‡] Timothy D. McKee, Robyn M. B. Loureiro, Barbara Tate, Weiming Xia,[§] Jeffrey L. Ives, Mark A. Findeis,[†] and Brian S. Bronk

Satori Pharmaceuticals, Inc., 281 Albany Street, Cambridge, Massachusetts 02139, United States

S Supporting Information

ABSTRACT: The discovery of a new series of γ-secretase modulators is disclosed. Starting from a triterpene glycoside γ-secretase modulator that gave a very low brain-to-plasma ratio, initial SAR and optimization involved replacement of a pendant sugar with a series of morpholines. This modification led to two compounds with significantly improved central nervous system (CNS) exposure.



Initial lead compound

Medicinal chemistry design strategies

Improved drug-like properties, CNS disposition

KEYWORDS: *Alzheimer's disease, amyloid β, γ-secretase, modulator, black cohosh*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the leading form of dementia and affects an estimated 5.4 million Americans[1] and 35.6 million people worldwide.[2] AD is the sixth leading cause of death in the United States, and with the aging baby boomer generation and increasing life expectancy, the prevalence and mortality rates associated with this disease are projected to rise dramatically. Healthcare costs associated with AD are already costing the United States government close to $200 billion annually, and recent estimates project that by 2050 the numbers of individuals afflicted with AD will increase to between 11 and 16 million in the United States[1] and 115.4 million people worldwide.[2] These cumulative social and economic factors make this one of the major healthcare crises facing the world today. Despite significant research efforts, there are currently no treatments which effectively slow or stop the progression of AD. Several symptomatic treatments have been approved, but these only temporarily improve symptoms of AD patients.[3,4] Thus, there is an urgent unmet medical need for a disease-modifying therapy for AD that can slow, halt, or reverse disease progression.

The pathology of the Alzheimer's brain is characterized by amyloid plaques and neurofibrillary tangles.[5−7] A prevailing viewpoint for the underlying cause of the disease is the amyloid hypothesis, which contends that amyloid β peptide (Aβ) dysregulation initiates a cascade of neuropathological changes—formation of amyloid plaques, neurofibrillary tangles, synaptic loss, and neurodegeneration—that ultimately result in the precipitous decline in cognition and ability to function in daily life that define AD dementia.[8] Amyloid plaques consist of Aβ peptides which are formed by the processing of amyloid precursor protein (APP). Aβ peptides are produced through a series of sequential cuts by two membrane-bound enzymes: first, β-secretase cleaves APP into the β-C-terminal fragment; then, γ-secretase makes further cuts to generate Aβ peptides ranging from 37 to 49 amino acids in length.[9,10] The main component of the amyloid plaques is the aggregation-prone 42 amino acid form of amyloid β (Aβ42), a relatively minor component in the total Aβ pool but particularly neurotoxic.[11−13] As a result, there has been much focus on both β-secretase and γ-secretase as therapeutic targets to interrupt the amyloid cascade by decreasing the amount of Aβ42 and thereby preventing the buildup of the amyloid plaques that initiate the disease.

Significant drug discovery research efforts have focused on inhibiting γ-secretase with small molecules to reduce the overall amount of amyloid production. Several different classes of these γ-secretase inhibitors (GSIs) have been reported in the literature and been shown to be potent inhibitors of γ-secretase activity, lowering Aβ levels *in vitro* and *in vivo*.[14−16] However, in addition to its role in the cleavage of APP, γ-secretase also cleaves multiple essential proteins, including Notch.[17] Thus, by inhibiting γ-secretase, GSIs also interfere with Notch processing, which leads to toxicities that include severe gastrointestinal abnormalities and skin cancer.[18,19] Several GSIs have been advanced to the clinic, but most clinical GSI studies have failed or been halted early due to observed toxicity, likely associated with the inhibition of Notch.[20,21]

In order to develop drugs with a safer profile, recent efforts have shifted from γ-secretase inhibition to γ-secretase modulation.[22−24] With the role of Aβ42 in the initiation of the disease process,[10] there is growing evidence that suggests it may be more important to lower the ratio of Aβ42/Aβ40 (Aβ40 being the most prevalent Aβ peptide residue) or Aβ42/total Aβ rather than reduce the total amount of Aβ.[25] γ-Secretase modulators (GSMs) achieve this profile by shifting

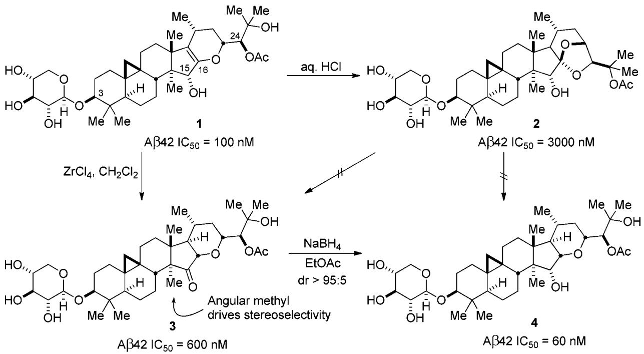
Special Issue: Alzheimer's Disease

Received: July 5, 2012
Accepted: August 29, 2012
Published: August 29, 2012

the sites of APP cleavage away from the more neurotoxic Aβ42 to shorter, nontoxic peptides such as Aβ37, Aβ38, and Aβ39. Importantly, GSMs have an added advantage in that they do not affect the release of the Notch intracellular domain (NICD) following the processing of Notch by γ-secretase, a vital factor in developing safe and tolerable therapeutics for AD.[26,27] The first reported GSMs were NSAIDs that were found to decrease the amounts of Aβ42 while increasing Aβ38.[28,29] A representative example is (R)-flurbiprofen, which advanced to clinical trials but failed due to lack of potency and poor brain exposure, a common problem among the NSAID GSMs.[30,31] There have been more recent disclosures of carboxylic acid GSMs and aryl imidazole GSMs which more potently reduce levels of Aβ42 while raising levels of Aβ38.[22,23] A recent report of three distinct pyrimidine-based GSMs showed reduction of both Aβ40 and Aβ42 *in vitro* but different effects on Aβ(37−39), with some examples raising Aβ38 and some having no effect on Aβ38 production.[32] These "second generation" GSMs have achieved much higher activities toward Aβ42 reduction than NSAIDs, and several examples have shown efficacy and selectivity both *in vitro* and *in vivo*, supporting modulation of γ-secretase as an effective approach to decreasing toxic species of Aβ without preventing the processing of other proteins, including Notch. Thus, modulation of γ-secretase has great potential as a therapeutic approach for the treatment of Alzheimer's disease.

As part of a program aimed at developing a series of novel γ-secretase modulators (GSMs) as therapeutics for AD, Satori identified an initial lead compound *via* screening of natural product extracts for selective reduction of Aβ42.[33] This initial lead (**1**, Scheme 1) was isolated from extracts of the black cohosh plant (*Actaea racemosa*) and represents the first entry in a unique class of GSMs that features a complex molecular architecture characterized by a plant sterol core structure. Most importantly, **1** exhibits a unique and compelling profile toward γ-secretase modulation, selectively reducing levels of Aβ42 and Aβ38 in vitro, while raising levels of Aβ37 and Aβ39. The levels of Aβ40 and total Aβ levels were maintained, and **1** also displayed pronounced selectivity over Notch processing *in vitro*.

Scheme 1



Additional Data:

Compound 1:
Aβ40 IC$_{50}$ = 2,700 nM; Aβ38 IC$_{50}$ = 61 nM; Aβ37,39 up
tPSA = 155; cLogP = 2.1; HBD = 5

Compound 4:
Aβ40 IC$_{50}$ = 1,000 nM; Aβ38 IC$_{50}$ = 84 nM
tPSA = 155; cLog P = 2.6; HBD = 5

While **1** was an intriguing initial hit, examination of its structure quickly revealed a number of potential metabolic and chemical liabilities that limited its potential usefulness as a therapeutic drug. In particular, the C3 glycoside, C16 enol ether, and C24 acetate stood out as areas of concern. Indeed, the concerns over the metabolic stability of **1** were confirmed upon dosing in CD1 mice. Rapid clearance of **1** precluded its therapeutic usefulness, as both the C3 sugar and the C24 acetate were readily metabolized *in vivo*.[33] In addition, the high MW and tPSA of **1** lay well outside the range typical for CNS drugs, causing concerns that we would not be able to prepare compounds that gave adequate CNS exposure to be therapeutically useful. Therefore, an important milestone in this project would be to prepare compounds that gave sufficient brain-to-plasma ratios *in vivo*.

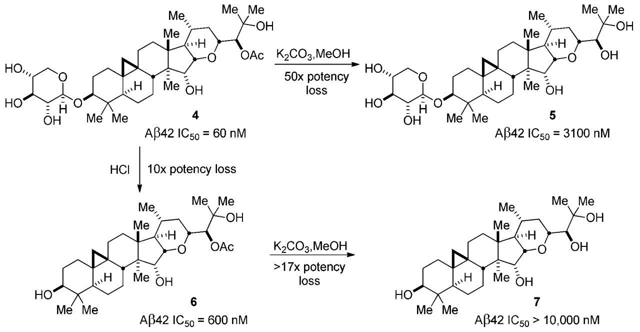
Recognizing the need to resolve the issues of intrinsic liability presented by the functionality native to this triterpene glycoside, the primary focus of initial medicinal chemistry efforts was placed on exploring the SAR around the core structure of the lead compound. Thus, we investigated the effects of removal or modification of selected structural features of **1** on the overall profile of modulation of Aβ processing by γ-secretase. In our initial exploratory forays into the chemistry of **1**, we exposed the compound to aqueous HCl, which resulted in acyl migration and subsequent formation of bicyclic ketal **2** and a 30-fold loss in potency toward Aβ42 reduction (Scheme 1). The bicyclic ketal was found to be quite stable and was resistant to a variety of reaction conditions, including many attempts to reopen the ketal. Thus, we turned our attention to exploring transformations that could be carried out on the enol ether and would not result in this irreversible cyclization. While many attempts at direct reduction of the C16−C17 olefin were unfruitful, we discovered that $ZrCl_4$ catalyzed the isomerization of the enol ether of **1** in CH_2Cl_2 to provide the C15 ketone **3** with *cis*-configuration between the C16 and C23 of the resulting *trans*-fused tetrahydropyran ring. While ketone **3** saw a 6-fold diminution of potency relative to **1**, subsequent treatment with $NaBH_4$ to reduce the C15 ketone and provide the corresponding C15 hydroxyl **4** moderately improved upon the potency of the initial lead (Aβ42 IC_{50} = 60 nM). The ketone reduction proceeded with excellent stereoselectivity (>95:5, only one product observed by ^1H NMR), with the angular methyl group at C14 presumably creating the steric environment that accounts for the high degree of selectivity in the reaction. The stereochemistry resulting from the two-step reduction process was confirmed by obtaining an X-ray crystal structure (see Supporting Information). Thus, in the process of removing one of the more troubling structural features from a metabolic and chemical perspective, the primary pharmacology of the lead compound was improved upon.

More extensive testing was carried out on **4**, and it was found to have excellent selectivity in transporter, off-target, and safety assays. However, following dosing in CD1 mice, the compound was found to have an insufficient pharmacokinetic profile for advancement, displaying moderate-to-high clearance and poor brain exposure (data not shown). This poor *in vivo* performance was not entirely unexpected, for the native C3 sugar appendage and the C24 acetate had been shown to be vulnerable to metabolism when **1** had been dosed *in vivo*.[33] In addition, little had been done to address other parameters, such as number of hydrogen bond donors (HBD = 5) and topological polar surface area (tPSA = 155 $Å^2$) that could preclude a molecule from achieving acceptable levels of brain exposure.[34−36] Thus, we sought to improve the overall CNS disposition by further modification of the structure of **4** that would address these design parameters.

Interrogation of the additional metabolic and chemical soft spots of **4** revealed some interesting trends in the SAR of this lead series. Hydrolysis of the C24 acetate to unmask the

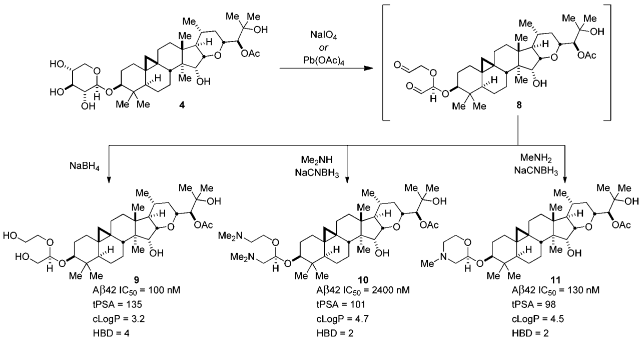
C24,C25 diol **5** resulted in a 50-fold reduction in potency in lowering Aβ42 (Scheme 2). Cleavage of the glycoside to provide triol **6** resulted in only a 10-fold loss of activity, while further treatment with K_2CO_3/MeOH to remove the C24 acetate and provide the tetrol **7** caused the potency to decrease by more than 17-fold compared to triol **6**. These results identified the C24 acetate as a more critical pharmacophore to the Aβ42 pharmacology than the C3 glycoside. As a result, the focus of these investigations shifted to carrying out further SAR studies at the C3 position of the scaffold through which we hoped to identify a glycoside surrogate that maintained the overall pharmacological profile while improving on the physicochemical properties of the series. These improvements would then hopefully translate into an improved pharmacokinetic profile.

Scheme 2



While part of our early work was focused on discovering a suitable replacement for the C3 glycoside *via* initial cleavage of the sugar followed by functionalization or chemical displacement of the exposed C3 alcohol,[37] we were also interested in exploiting rather than replacing the native sugar in our synthetic endeavors. To this aim, by treating **4** with $NaIO_4$ or $Pb(OAc)_4$, we were able to carry out a double oxidative cleavage event on the sugar to provide dialdehyde **8** (Scheme 3), which served as a versatile intermediate.[38,39] Reduction of dialdehyde **8** with $NaBH_4$ provided tetrol substrate **9**, which possessed good pharmacology but did not offer any advantages over **4** from a physicochemical properties standpoint. Dual reductive amination with dimethylamine provided the diamine **10**, which, although lowering the tPSA, also realized a diminution in potency. When we carried out the reductive amination of dialdehyde **8** with methylamine, after undergoing an initial reductive amination, the intermediate amino-aldehyde species participated in a second intramolecular reductive amination event to provide C3 morpholine **11**. This two-step chemical transformation of the native sugar into a morpholine provided a substrate which not only maintained the primary pharmacology (Aβ42 IC_{50} = 130 nM) but offered a significant improvement from a physicochemical properties perspective, lowering the tPSA to 98 Å^2 and the HBD count to two. Thus, by unlocking the reactive potential of the 1,2,3-triol of the sugar to synthesize the C3 morpholine as a replacement for the C3 glycoside, we had discovered a new lead series of compounds possessing a modified headpiece which maintained high potency in lowering Aβ42 *in vitro* and provided a versatile handle for further derivatization to allow for engineering of the overall physicochemical properties.

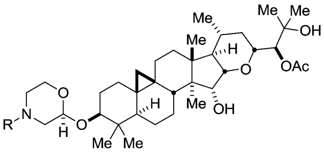
Scheme 3

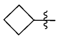
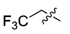
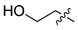
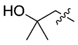
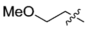
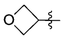
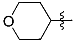
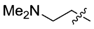
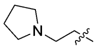
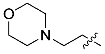
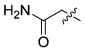
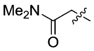
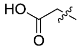
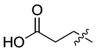
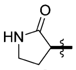
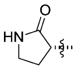
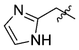
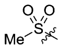
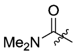
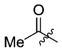
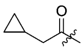
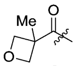
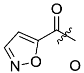
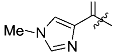


To fully exploit the medicinal and synthetic chemistry potential of this C3 morpholine series of compounds, a focused but diverse range of morpholine compounds was designed and synthesized, and representative examples from this series of compounds are shown in Table 1. A scan of the SAR of this C3 morpholine series reveals that a wide variety of substitution is tolerated on the morpholine nitrogen. The simple N−H morpholine **12** was particularly potent with an Aβ42 IC_{50} of 70 nM. Small aliphatic substituents were also tolerated, and the general trends suggest that polar substituents on a basic morpholine are preferred over more lipophilic substituents, a trend that was in concert with one of our key design elements. Hence, while N-methyl (**11**) and N-ethyl morpholines (**13**) maintained potency, imparting more lipophilic character by functionalization of the morpholine nitrogen with propyl, benzyl, or cycloalkyl groups resulted in a loss of activity (**14**−**17**). A decrease in potency was also realized for *N*-trifluoroethyl morpholine **18**. Incorporating a heteroatom into the morpholine substituents and thereby increasing the polarity in this region of the molecule resulted in improved Aβ42 activity relative to the purely aliphatic analogs. For example, replacing the terminal methyl of the *N*-propyl derivative **14** with a hydroxyl (**19**) or a methyl ether (**21**) resulted in much improved potency, and the *N*-oxetane derivative **22** was 2-fold more potent than the corresponding *N*-cyclobutyl analog **17**. Both hydroxyalkyl and ether appendages on the morpholine nitrogen showed a strong propensity for reduction of Aβ42 (**19**−**23**). A similar effect was seen upon incorporation of aminoalkyls into the morpholine side chain, providing compounds with strong Aβ42 lowering capabilities (**24**−**26**). Amide appendages on the morpholine nitrogen also resulted in extremely potent compounds (**27**−**28**), but carboxylic acids were not as active (**29**−**30**). Both diastereomers of the γ-lactam moiety showed good activity (**31** and **32**), as did the imidazole derivative **33**. Whether or not the improved potency results from the ability of the heteroatoms to pick up additional interactions or is simply due to increased polarity is not clear.

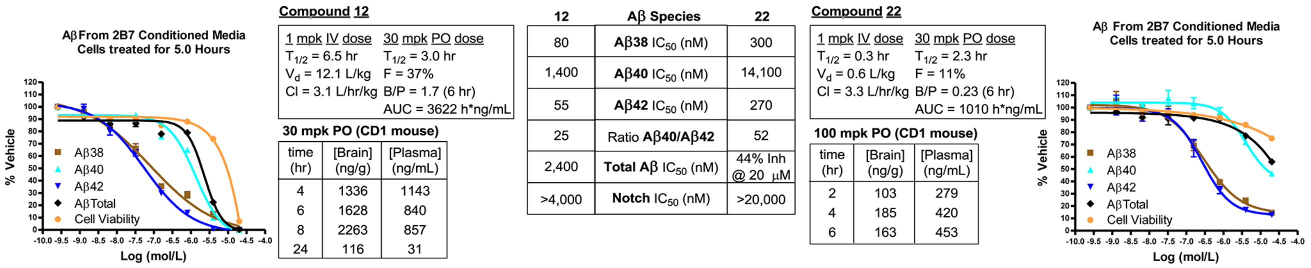
The synthesis of acyl morpholines and related species also furnished interesting and highly potent analogs. Conversion of the morpholine nitrogen to sulfonamide **34**, urea **35**, and amide derivatives (**36**−**40**) provided a series of compounds with excellent activity toward Aβ42 lowering. Although these acylated derivatives were intriguing, the physicochemical properties of these compounds were less compelling than morpholines that maintained a basic center (Supporting Information). The morpholine headpiece proved to be a very versatile handle for incorporating a diverse range of functional groups into the scaffold, and it proved advantageous for tuning the overall molecular properties and the potential to improve

Table 1. SAR of Representative Examples from the Satori C3 Morpholine Series of GSMs



Compound	R	Aβ42 IC_{50} (nM)[a]	Aβ40 IC_{50} (nM)/ % reduction
11	Me	130	3,400
12	H	70	2,300
13	Et	190	42% @ 4 uM
14	Pr	680	15,900
15	Bn	2,000	4% @ 4 uM
16		1,000	35% @ 20 uM
17		350	44% @ 4 uM
18		1,190	47% @ 20 uM
19		240	46% @ 4 uM
20		230	9,400
21		210	7,600
22		170	41% @ 20 uM
23		180	38% @ 4 uM
24		90	29% @ 0.8 uM
25		110	34% @ 0.8 uM
26		160	49% @ 4 uM
27		150	7,800
28		140	9,000
29		370	14,000
30		490	18,800
31		200	7,000
32		120	44% @ 20 uM
33		120	38% @ 4 uM
34		80	40% @ 20 uM
35		60	40% @ 20 uM
36		90	13,400
37		130	38% @ 20 uM
38		80	8,000
39		70	4,800
40		130	44% @ 20 uM

[a] IC_{50} reported as an average of multiple determinations ($n \geqslant 2$).



Compound 12

1 mpk IV dose	30 mpk PO dose
$T_{1/2}$ = 6.5 hr	$T_{1/2}$ = 3.0 hr
V_d = 12.1 L/kg	F = 37%
Cl = 3.1 L/hr/kg	B/P = 1.7 (6 hr)
	AUC = 3622 h*ng/mL

30 mpk PO (CD1 mouse)

time (hr)	[Brain] (ng/g)	[Plasma] (ng/mL)
4	1336	1143
6	1628	840
8	2263	857
24	116	31

12	Aβ Species	22
80	**Aβ38** IC_{50} (nM)	300
1,400	**Aβ40** IC_{50} (nM)	14,100
55	**Aβ42** IC_{50} (nM)	270
25	Ratio **Aβ40/Aβ42**	52
2,400	**Total Aβ** IC_{50} (nM)	44% Inh @ 20 μM
>4,000	**Notch** IC_{50} (nM)	>20,000

Compound 22

1 mpk IV dose	30 mpk PO dose
$T_{1/2}$ = 0.3 hr	$T_{1/2}$ = 2.3 hr
V_d = 0.6 L/kg	F = 11%
Cl = 3.3 L/hr/kg	B/P = 0.23 (6 hr)
	AUC = 1010 h*ng/mL

100 mpk PO (CD1 mouse)

time (hr)	[Brain] (ng/g)	[Plasma] (ng/mL)
2	103	279
4	185	420
6	163	453

Figure 1. Full Aβ production and pharmacokinetic profiles (CD1 mouse) for compounds **12** and **22**.

the CNS disposition. In addition, the selectivity for reduction of Aβ42 versus Aβ40 was maintained across the series.

The overall profile of two C3 morpholine derivatives, N−H morpholine **12** and the N-oxetane analog **22**, allowed these to emerge as candidates for further evaluation. While all molecules had a higher molecular weight than is usually targeted for a CNS drug, the balance of potency, HBD, tPSA, and lipophilicity of analogs **12** and **22** along with the potential benefits provided by having a basic center in the molecule to assist in permeating the blood−brain barrier elevated these over our initial leads (**1** and **4**) and other potential candidates in the series (Supporting Information).

The C3 morpholines **12** and **22** were dosed in CD1 mice to evaluate *in vivo* pharmacokinetics (Figure 1). The results showed that attenuating amine basicity in *N*-oxetane morpholine species **22** (pK_a = 4.6) decreases the volume of distribution (0.6 L/kg versus 12.1 L/kg for **12**) while modestly increasing the clearance (3.3 L/(h kg) for **22** versus 3.1 L/(h kg)) compared to the more basic N−H morpholine **12** (pK_a = 7.7). Most importantly, we found that these compounds gave encouraging brain-to-plasma ratios (1.7 for **12**, 0.23 for **22**). While the compounds exhibited acceptable brain-to-plasma ratios, the bioavailability values were low (%*F* = 11% for **22** and 37% for **12**, respectively) and low levels of brain exposure were observed. Still, this murine PK data represented a significant improvement over the previously disclosed lead compounds from the C3 glycosidic series of GSMs (**1** and **4**), and we felt this disposition and overall profile were sufficient to assess a PK/PD response *in vivo*.

After comparing the *in vivo* PK and predicted microsomal stability of **12** versus **22** in mouse (MLM, % remaining after 60 min ± NADPH: **12** = 20%/25%; **22** = 1%/1%), we decided to focus on **12**, which was taken forward for *in vivo* efficacy evaluation in CD1 mice.[40] Unfortunately, due to the high clearance of the molecules, we were unable to obtain sufficient brain exposure to elicit a robust pharmacodynamic response *in vivo* (see Supporting Information). Results of further studies aimed at improving clearance of this series in order to obtain adequate exposure for a PD response will be presented in due course.

Evaluation of the Aβ profile in our cell-based assay showed that both N−H morpholine **12** and *N*-oxetane analog **22** exhibited the ability to lower Aβ42 levels in cells, with similar activity versus Aβ38, good selectivity for lowering Aβ42 versus Aβ40 (ratio of Aβ40 IC_{50}/Aβ42 IC_{50} = 25 for **12** and 52 for **22**), and preserved levels of total Aβ in cells[42] (Figure 1). The molecules showed excellent selectivity for lowering Aβ42 versus Notch processing (>70-fold selective for both **12** and **22**). This level of Notch selectivity is consistent with a GSM profile and, due to the toxicities associated with inhibition of Notch processing, offers a distinct advantage over the lack of Notch selectivity exhibited by GSIs which have advanced to clinical trials.[43] The overall modulation profile of the series, reducing Aβ42 and Aβ38 while maintaining Aβ40 and total Aβ, represents a different GSM profile when compared to most other GSMs reported in the literature.

In summary, the Satori GSM program progressed from an intriguing initial hit, triterpene glycoside **1**, identified in a targeted bioassay screening, to a new lead compound (**4**) with a modified core. Application of medicinal chemistry design principles in conjunction with innovative synthetic chemistry approaches led to the discovery of a new series of C3 morpholine compounds. This series represents a new structural class of GSMs that maintained the compelling pharmacological profile of the initial leads, lowering Aβ42 and Aβ38 levels in cells, preserving Aβ40 and total Aβ, and showing no inhibition of Notch activity. This GSM profile differs from most other GSMs reported in the literature, which lower Aβ42 but raise levels of Aβ38. In the process of design, we significantly improved druglike properties of the lead compounds by decreasing both the tPSA and the overall count of hydrogen bond donors (HBD) while keeping the lipophilicity in an acceptable range (<5). This led to compounds **12** and **22**, which have significantly improved CNS penetration relative to glycoside **4**, which more closely resembles our natural product lead **1**. In addition, these compounds retained an excellent profile in standard transporter, off-target, and safety assays *in vitro*, and showed improved chemical stability. The morpholine nitrogen provides a convenient and versatile handle by which the overall molecular properties of the compounds can be readily manipulated. The discovery of this new series of C3 morpholine GSMs with a plant sterol-derived core structure represents a novel and exciting entry into the arena of γ-secretase modulation as a potential therapeutic approach for Alzheimer's disease.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Calculated physicochemical properties of high-interest compounds from the Satori C3 morpholine series of GSMs, exemplary procedures for the synthesis of compounds of interest, characterization data of key compounds **12** and **22**, X-ray crystal structure of compound **4**, and assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 617-547-0022. Fax: 617-547-0661. E-mail: nathan.fuller@satoripharma.com.

Present Addresses

[†]Resilientx Therapeutics, 431 School Street, Belmont, MA 02478, USA.
[‡]Genzyme Corporation, 500 Kendall Street, Cambridge, MA 02142.
[§]Department of Veterans Affairs, Boston, MA.

Author Contributions

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

Notes

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.

■ ACKNOWLEDGMENTS

The authors would like to thank Ruichao Shen for his comments on the paper.

■ ABBREVIATIONS

Aβ, amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein; cLogP, calculated lipophilicity; CH_2Cl_2, dichloromethane; CNS, central nervous system; EtOAc, ethyl acetate; GSM, γ-secretase modulator; GSI, γ-secretase inhibitor; HCl, hydrochloric acid; HBD, hydrogen bond donors; K_2CO_3, potassium carbonate; Me_2NH, dimethylamine; $MeNH_2$, methylamine; MLM, mouse liver microsomes; MeOH, methanol; $NaBH_4$, sodium borohydride; $NaCNBH_3$, sodium cyanoborohydride; $NaIO_4$, sodium periodate; NSAID, nonsteroidal anti-inflammatory drug; $Pb(OAc)_4$, lead tetraacetate; PK, pharmacokinetic; PO, by mouth; tPSA, topological polar surface area; SAR, structure−activity relationship; $ZrCl_4$, zirconium tetrachloride

■ REFERENCES

(1) Alzheimer's Association. Alzheimer's Disease Facts and Figures. *Alzheimer's Dementia* **2012**, *8* (22), 131−168 (http://www.alz.org/downloads/Facts_Figures_2012.pdf).

(2) Wimo, A. P. Alzheimer's Disease International, World Alzheimer's Report 2010: The Global Economic Impact of Dementia, 21 September, 2010.

(3) *Alzheimer's Disease Medications Fact Sheet*, NIH Publication No. 08-3431; Department of Health and Human Services, National Institute on Aging, U.S. Government Printing Office: Washington, D.C., 2010.

(4) Cummings, J. L. *N. Engl. J. Med.* **2004**, *351*, 56.

(5) Serrano-Pozo, A.; Frosch, M. P.; Masliah, E.; Hyman, B. T. Neuropathological Alterations in Alzheimer Disease. In *The Biology of Alzheimer Disease*; Selkoe, D. J., Mandelkow, E., Holtzman, D. M., Eds.; Cold Spring Harbor Perspectives Laboratory Press: Cold Spring Harbor, NY, 2012; pp 43−65.

(6) Terry, R. D. Alzheimer's Disease at mid century (1927−1977). In *Alzheimer: 100 Years and Beyond*; Jucker, M., Beyreuther, K., Haass, C., Nitsch, R., Eds.; Springer-Verlag: Berlin Heidelberg, 2006; pp 58−61.

(7) Holtzman, D. M.; Morris, J. C.; Goate, A. M. Alzheimer's disease: The challenge of the second century. *Sci. Transl. Med.* **2011**, *3*, 77sr1.

(8) Hardy, J.; Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **2002**, *297* (5580), 353−356.

(9) Haass, C.; Kaether, C.; Thinakaran, G.; Sisodia, S. Trafficking and Proteolytic Processing of APP. In *The Biology of Alzheimer Disease*; Selkoe, D. J., Mandelkow, E., Holtzman, D. M., Eds.; Cold Spring Harbor Perspectives Laboratory Press: Cold Spring Harbor, NY, 2012; pp 205−229.

(10) Selkoe, D. J. Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* **2001**, *81*, 741−766.

(11) Roher, A. E.; Lowenson, J. D.; Clarke, S.; Woods, A. S.; Cotter, R. J.; Gowing, E.; Ball, M. J. β-Amyloid-(1−42) is a major component of cerebrovascular amyloid deposits: Implications for the pathology of

Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10836–10840.

(12) Iwatsubo, T.; Odaka, A.; Suzuki, N.; Mizusawa, H.; Nukina, N.; Ihara, Y. Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42(43). *Neuron* **1994**, *13*, 45–53.

(13) Findeis, M. A. The role of amyloid β peptide 42 in Alzheimer's disease. *Pharmacol. Ther.* **2007**, *116*, 266–286.

(14) Kreft, A. F.; Martone, R.; Porte, A. Recent advances in the identification of gamma-secretase inhibitors to clinically test the Abeta oligomer hypothesis of Alzheimer's disease. *J. Med. Chem.* **2009**, *52*, 6169–6188.

(15) Panza, F.; Frisardi, V.; Imbimbo, B. P.; Capurso, C.; Logroscino, G.; Sancarlo, D.; Seripa, D.; Vendemiale, G.; Pilotto, A.; Solfrizzi, V. γ-Secretase inhibitors for the treatment of Alzheimer's disease: the current state. *CNS Neurosci. Ther.* **2010**, *16* (5), 272–284.

(16) D'Onofrio, G.; Panza, F.; Frisardi, V.; Solfrizzi, V.; Imbimbo, B. P.; Paroni, G.; Cascavilla, L.; Seripa, D.; Pilotto, A. Advances in the identification of γ-secretase inhibitors for the treatment of Alzheimer's disease. *Expert Opin. Drug Discovery* **2012**, *7*, 19–37.

(17) Lleo, A. Activity of γ-secretase on substrates other than APP. *Curr. Top. Med. Chem.* **2008**, *8*, 9–16.

(18) Searfoss, G. H.; Jordan, W. H.; Calligaro, D. O.; Galbreath, E. J.; Schirtzinger, L. M.; Berridge, B. R.; Gao, H.; Higgins, M. A.; May, P. C.; Ryan, T. P. Adipsin, a Biomarker of Gastrointestinal Toxicity Mediated by a Functional γ-Secretase Inhibitor. *J. Biol. Chem.* **2003**, *278*, 46107–46116.

(19) Wong, G. T.; Manfra, D.; Poulet, F. M.; Zhang, Q.; Josien, H.; Bara, T.; Engstrom, L.; Pinzon-Ortiz, M.; Fine, J. S.; Lee, H. J.; Zhang, L.; Higgins, G. A.; Parker, E. M. Chronic Treatment with the γ-Secretase Inhibitor LY-411575 Inhibits β-Amyloid Peptide Production and Alters Lymphopoiesis and Intestinal Cell Differentiation. *J. Biol. Chem.* **2004**, *279*, 12876–12882.

(20) Kreft, A. F.; Martone, R.; Porte, A. Recent advances in the identification of γ-secretase inhibitors to clinically test the Aβ oligomer hypothesis of Alzheimer's disease. *J. Med. Chem.* **2009**, *52*, 6169–6188.

(21) Hopkins, C. R. ACS Chemical Neuroscience Molecule Spotlight on Semagacestat (LY450139). *ACS Chem. Neurosci.* **2010**, *1*, 533–534.

(22) Oehlrich, D.; Berthelot, D. J.-C.; Gijsen, H. J. M. γ-Secretase Modulators as Potential Disease Modifying Anti-Alzheimer's Drugs. *J. Med. Chem.* **2011**, *54*, 669–698.

(23) Wolfe, M. S. γ-Secretase inhibitors and modulators for Alzheimer's disease. *J. Neurochem.* **2012**, *120* (1), 89–98.

(24) Wolfe, M. S. γ-Secretase: Structure, Function, and Modulation for Alzheimer's Disease. *Curr. Top. Med. Chem.* **2008**, *8*, 2–8.

(25) Karran, E.; Mercken, M.; De Strooper, B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat. Rev. Drug Discovery* **2011**, *10*, 698–712.

(26) Wanngren, J.; Ottervald, J.; Parpal, S.; Portelius, E.; Strömberg, K.; Borgegård, T.; Klintenberg, R.; Juréus, A.; Blomqvist, J.; Blennow, K.; Zetterberg, H.; Lundkvist, J.; Rosqvist, S.; Karlström, H. Second generation γ-secretase modulators exhibit different modulation of Notch β and Aβ production. *J. Biol. Chem.* **2012**, article in press.

(27) Okochi, M.; Fukumori, A.; Jiang, J.; Itoh, N.; Kimura, R.; Steiner, H.; Haass, C.; Tagami, S.; Takeda, M. Secretion of the Notch-1 Aβ-like Peptide during Notch Signaling. *J. Biol. Chem.* **2006**, *281*, 7890–7898.

(28) Weggen, S.; Erikson, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Butler, T.; Kang, D. E.; Marquez-Sterling, N.; Golde, T. E.; Koo, E. H. A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. *Nature* **2001**, *414*, 212–216.

(29) Erikson, J. L.; Sagi, S. A.; Smith, T. E.; Weggen, S.; Das, P.; McLendon, D. C.; Ozols, V. V.; Jessing, K. W.; Zavitz, K. H.; Koo, E. H.; Golde, T. E. NSAIDs and enantiomers of flurbiprofen target γ-secretase and lower Aβ42 in vivo. *J. Clin. Invest.* **2003**, *112*, 440–449.

(30) Galasko, D. R.; Graff-Radford, N.; May, S.; Hendrix, S.; Cottrell, B. A.; Sagi, S. A.; Mather, G.; Laughlin, M.; Zavitz, K. H.; Swabb, E.; Golde, T. E.; Murphy, M. P.; Koo, E. H. Safety, tolerability, pharmacokinetics, and Aβ levels after short-term administration of R-flurbiprofen in healthy elderly individuals. *Alzheimer's Dis. Assoc. Disord.* **2007**, *21*, 292–299.

(31) Green, R. C.; Schneider, L. S.; Amato, D. A.; Beelen, A. P.; Wilcock, G.; Swabb, E. A.; Zavitz, K. H.; Tarenflurbil Phase 3 Study Group. Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: a randomized controlled trial. *J. Am. Med. Assoc.* **2009**, *302*, 2557–2564.

(32) Borgegard, T.; Jeréus, A.; Olsson, F.; Rosqvist, S.; Sabirsh, A.; Rotticci, D.; Paulsen, K.; Klintenberg, R.; Yan, H.; Waldman, M.; Stromberg, K.; Nord, J.; Johansson, J.; Regner, A.; Parpal, S.; Malinowsky, D.; Radesater, A.-C.; Li, T.; Singh, R.; Eriksson, H.; Lundkvist, J. First and Second Generation γ-Secretase Modulators (GSMs) Modulate Amyloid-β (Aβ) Peptide Production through Different Mechanisms. *J. Biol. Chem.* **2012**, *287*, 11810–11819.

(33) Findeis, M. A.; Schroeder, F.; McKee, T. D.; Yager, D.; Fraering, P. C.; Creaser, S. P.; Austin, W. F.; Clardy, J.; Wang, R.; Selkoe, D. J.; Eckman, C. B. Discovery of a novel pharmacological and structural class of gamma secretase modulators derived from the extract of *Actaea racemosa*. *ACS Chem. Neurosci.* DOI: 10.1021/cn3000857; manuscript in press.

(34) Wager, T. T.; Hou, X.; Verhoest, P. R.; Villalobos, A. Moving beyond Rules: The Development of a Central Nervous System Multiparameter Optimization (CNS MPO) Approach To Enable Alignment of Druglike Properties. *ACS Chem. Neurosi.* **2010**, *1*, 435–449.

(35) Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. Defining Desirable Central Nervous System Drug Space through the Alignment of Molecular Properties, in Vitro ADME, and Safety Attributes. *ACS Chem. Neurosci.* **2010**, *1*, 420–434.

(36) Lipinski, C. A.; Lombardo, R.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.

(37) Austin, W. F.; Hubbs, J. L.; Fuller, N. O.; Creaser, S. P.; McKee, T. D.; Loureiro, R. M. B.; Findeis, M. A.; Tate, B.; Ives, J. L.; Bronk, B. S. SAR investigations on a novel class of gamma-secretase modulators based on a unique scaffold. (Manuscript submitted for publication).

(38) Du, M.; Hindsgaul, O. Novel Hybrid Morpholino-Glycopeptides with the Amino Acid Nitrogen Replacing C-3 of the Pyranose Ring. *Synlett* **1997**, 395–397.

(39) Chan, J. Y. C.; Hough, L.; Richardson, A. C. J. The Synthesis of (R)- and (S)-Spirobi-1,4-dioxane and Related Spirobicycles from D-Fructose. *J. Chem. Soc., Perkin. Trans. 1* **1985**, *7*, 1457–1462.

(40) For results and protocol for *in vitro* profiling for microsomal stability in human, mouse, and rat, see the Supporting Information.

(41) The pK_a, ClogP, and tPSA predictions in this paper were calculated using algorithms included in MarvinSketch, version 5.4.1.1; ChemAxon: Budapest, Hungary, 2011.

(42) When dosed at higher concentrations (between 1 and 4 μM), compound **12** begins to show some reduction in cell viability (20% reduction @ 4 μM, Cell Titre Glo assay), and the Aβ40 and total Aβ levels are lowered at these higher concentrations as a result of reduced cell viability. Compound **22** shows only slight cell viability issues at the higher end of the concentration spectrum tested (20% reduction @ 20 μM).

(43) Known GSIs which have advanced to clinical trials show no selectivity for Notch inhibition (ratio of Aβ IC$_{50}$/Notch IC$_{50}$ for semagecestat = 0.6, for begacestat = 0.8, and for avagecestat = 0.6. Known GSMs in the literature show much better selectivity toward Notch inhibition (Aβ42 IC$_{50}$/Notch IC$_{50}$ for Merck GSM-1 = >350, for JNJ-40418677 = >20, for Eisai E-2012 = >400). See "An improved cell-based method for determining the gamma-secretase enzyme activity against both NOTCH and APP substrates", T. D. McKee, et al., AAIC 2012 poster #P2-095, http://www.satoripharma.com/news-events/publications-and-presentations.php.