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Synthesis and evaluation of succinoyl-caprolactam γ-secretase inhibitors

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Dedicated to the memory of David W. Robertson, mentor, colleague and friend.

Abstract—The synthesis, evaluation, and structure–activity relationships of a series of succinoyl lactam inhibitors of the Alzheimer's disease γ-secretase are described. Beginning with a screening hit with broad proteinase activity, optimization provided compounds with both high selectivity for inhibition of γ-secretase and high potency in cellular assays of Aβ reduction. The SAR and early in vivo properties of this series of inhibitors will be presented.

Alzheimer's disease (AD) is the leading cause of dementia and is currently believed to affect 4 million Americans and up to 30 million people worldwide.[1] In addition to the devastating burden of the disease on patients and their families, estimates of the financial cost of the disease range up to $100 billion annually.[2] Current treatments include acetylcholinesterase inhibitors and the NMDA blocker memantine; however, these therapies treat disease symptoms and do not significantly affect the underlying progression of the disease. AD pathology is characterized by the accumulation in the brain of extracellular amyloid plaques composed largely of the β-amyloid peptide (Aβ) and intracellular neurofibrillary tangles composed of hyperphosphorylated Tau protein. Most strategies to develop disease-modifying treatments center on inhibiting the formation of these lesions. Genetic evidence obtained from familial forms of AD suggests that increased production of the 42 amino acid form of Aβ has a primary role in the disease.[1]

The Aβ peptides are generated by successive cleavages of the amyloid precursor protein (APP) by β- and γ-secretases which have emerged as strong therapeutic targets for AD intervention.[3–5] β-Secretase (typically referred to as BACE1) is a novel membrane-embedded aspartyl proteinase whose characterization was reported by a number of groups in late 1999.[6–10] γ-Secretase is now known to consist of a complex of four proteins: presenilin 1 or 2, nicastrin, Pen-2, and Aph-1, with

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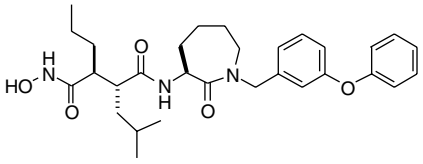
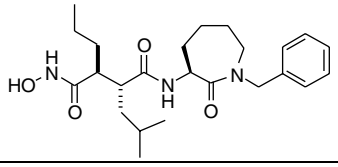
presenilin considered to be the likely catalytic component.[11–13] Pharmacological blockade of γ-secretase inhibits the production of Aβ in transgenic models of Alzheimer's disease.[14,15]

Based on the therapeutic potential of inhibiting Aβ production, we initiated a program designed to identify novel inhibitors of the secretases. High-throughput screening using a cellular assay[16] for inhibition of Aβ production revealed several hydroxamic acid-based inhibitors with promising activity (Table 1). The most potent hit identified was SR973 (**1**), an aminocaprolactam succinate derivative which blocks Aβ formation with an IC_{50} = 0.2 μM. A small set of related derivatives were also evaluated, with the parent benzyl caprolactam (Table 1, compound **2**) some 30 times less potent. The more polar 4-nitro and 4-amino substituted compounds were inactive. This early SAR suggested that the nature of the caprolactam N-substituent was an important contributor to activity, with preference for a fairly large hydrophobic substituent at this position.

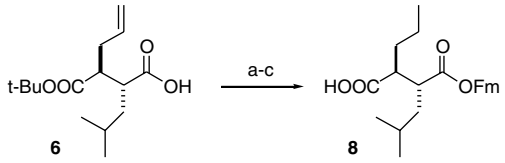
In order to gain basic SAR in this series, a flexible solid-phase synthesis was designed. Attachment of intermediates such as **9** (Scheme 2) to resin through the hydroxamic acid provided a straightforward way to modify the caprolactam *N*-substituent. As commercially available trityl hydroxylamine resin is cleaved with mild acid, we chose to block the second carboxylate of the succinate as the orthogonal 9-fluorenylmethyl (Fm) ester.

The synthesis of *anti*-succinate **6** (Scheme 1) with good diastereocontrol has been reported.[17] Reduction of the allyl group, protection of the free carboxylate as the Fm ester, and deprotection of the *t*-butyl ester produced the protected succinate **8** (Scheme 1). As a first set of targets, the SAR of the caprolactam amine substituent was explored by preparing a set of 3-substituted biaryl derivatives, which probed the space of the aryloxy substituent with a more rigid framework. This strategy required the synthesis of 3-iodobenzyl aminocaprolactam, which was simply prepared by deprotonation of (*S*)-*N*-boc-aminocaprolactam with LDA followed by alkylation with 3-iodobenzyl bromide and deprotection.

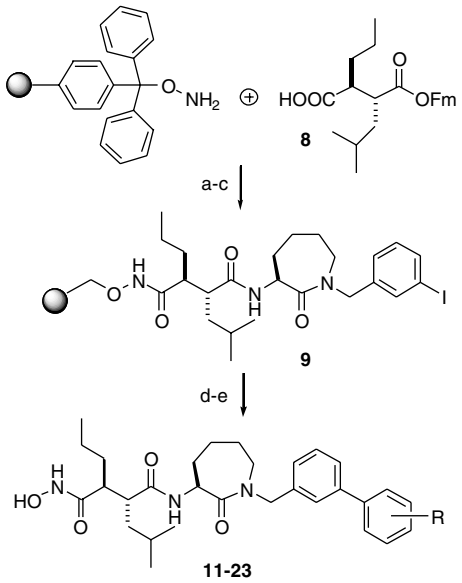
Table 1. Succinyl hydroxamate Aβ production inhibitor leads

Compound	Structure	IC_{50}[a] (nM)
1 (SR973)		200
2		6800

[a] Activity assayed in CHO N9 cells according to Ref. 16.



Scheme 1. Reagents and conditions (Fm = 9-fluorenylmethyl): (a) 5% Pd/C, H_2; (b) FmOH, DCC, DMAP, DCM, rt; (c) 50% TFA/DCM, rt.



Scheme 2. Reagents and conditions (Fm = 9-fluorenylmethyl): (a) HATU, DIEA, DMF, rt; (b) 25% piperidine in DMF, repeat; (c) caprolactam, PyBOP, DIEA, DMF, rt, repeat; (d) Boronic acid, $Pd(PPh_3)_4$, THF, 2 M Na_2CO_3, 60 °C, 16 h; (e) 50% TFA/DCM, rt.

Solid-phase synthesis of substituted biaryls was carried out according to the procedure in Scheme 2. Fmoc-protected trityl hydroxamate resin was deprotected and acylated with succinate **8**. Deprotection of the Fm ester and spectrophotometric quantitation allowed accurate determination of the succinate loading level. Subsequent coupling with the substituted aminocaprolactam provided the precursor **9** for biaryl formation via a Suzuki coupling protocol.[18] Cleavage from the resin with TFA and purification on a short silica plug returned 6–13 mg (14–60%) of each compound with no optimization of the sequence. The compounds were isolated in high purity, however the yields varied due to cleavage from the solid support during the Suzuki coupling.

Examination of the SAR in this series (Table 2) showed that truncation of the 3-substituent to the parent 3-iodo compound **11** caused a 6-fold reduction in potency relative to the phenyl ether **2** (500 nM, see Table 4). Biaryls with a single substituent in the 4-position had potencies in the 250–500 nM range. Potency was not particularly sensitive to electronic effects (compare compounds **13** and **15**). Substituents at the 2- and 3-position gave potencies in the same range with a trend toward 3-halo substituents being preferred. The polar 3-acetamido group was not tolerated. The 4-fluoro-3-chloro-substituted analog **17** was the most potent in the series at 100 nM, a modest 5-fold improvement relative to compound **2**.

Table 2. Activity of selected biaryl succinyl hydroxamates **11–23**

Compound	Substituent	IC_{50}[a] (nM)
11	Parent 3- iodobenzyl (Cleavage of 9)	3,000
12	2,4-Dichloro	350
13	4-Fluoro	470
14	4-Methyl	260
15	4-Methoxy	470
16	3-Methyl	510
17	4-Fluoro, 3-chloro	100
18	4-Trifluoro methyl	230
19	3-Methoxy	600
20	3-Acetamido	>10,000
21	3-Fluoro	180
22	2-Methoxy	300
23	2-(l-Naphthyl)	250

[a] Activity assayed in HEK 293 cells according to Ref. 16.

Concurrent work examined the role of the hydroxamic acid in this series. The original lead, SR973 (**1**), had previously been profiled as an inhibitor of the ADAM (a disintegrin and metalloproteinase) family of enzymes as part of a program targeting inhibitors of both MMPs and the tumor necrosis factor α-converting enzyme (TACE, or ADAM 17).[19–21] SR973 is a potent, broad spectrum MMP inhibitor, with nanomolar activity against MMPs and TACE (Table 3). This observation raised the possibility that γ-secretase might be a related metalloproteinase, which could make achieving selectivity versus the other MMPs difficult. We therefore explored this hypothesis structurally by modification of the carboxy-terminal group on the molecule.

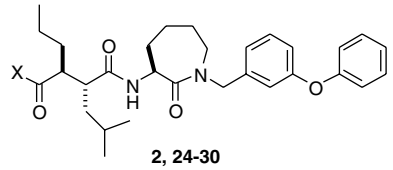
The requisite carboxylic acid **24** was constructed by coupling the alkylated caprolactam to succinate **6** (PyBOP, DIEA, and DMF) followed by hydrogenation of the allyl group and TFA-mediated cleavage of the *t*-butyl ester. As is shown in Table 4, O- or N-methylation of the hydroxamic acid caused a 10-fold loss in affinity. While significant, this potency loss is not as severe as might be expected for disrupting the interaction of a hydroxamic acid binding to a zinc metalloproteinase.[22] Similar results were obtained for other small groups including the methyl amide and methyl ester.[29] Surprisingly, the primary amide **30** (RE987, IC_{50} = 50 nM) was 10-fold more potent than the hydroxamate **1** and was inactive against a panel of MMPs and several serine proteases (data not shown). Taken together, these results are inconsistent with the target of these compounds being a metalloproteinase. Recent data suggest that the presenilins are the catalytic component of the γ-secretase

Table 3. SR973 potency at metalloproteinases

Enzyme	K_i (nM)
MMP-1	21
MMP-2	7
MMP-7	660
MMP-9	5
MMP-10	190
MMP-13	10
MMP-14	1200
TACE	4

Activity assayed according to Ref. 17.

Table 4. Succinyl carboxylate modifications



2, 24-30

Compound	Substituent (X group, structure xx)	IC_{50}[a] (nM)
1 (SR973)	NH-OH	500
24	OH	>3000
25	NH-OCH	5000
26	$N(Me)OCH_3$	5000
27	$NHCH_3$	5000
28	OCH_3	6000
29	$NHNH_2$	1600
30 (RE987)	NH_2	50

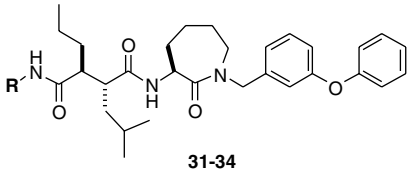
[a] Activity assayed in HEK 293 cells according to Ref. 16.

complex. These proteins contain two membrane-embedded aspartyl residues that are essential to catalytic function.[23–26] Homology between the putative active site of the presenilins and the bacterial type-4 prepilin peptidases, which are also aspartyl proteases, provides additional support for this hypothesis.[27]

Based on the potency of the primary amide **30**, additional amide variants were tested. This was accomplished by reductively loading amines onto BAL[28] resin followed by coupling to acid **24**. Cleavage from the washed solid support provided clean products in excellent yields. Of 24 analogs, the 4 most potent compounds had modest activity (in the 3–6 μM range, Table 5), demonstrating that high potency in this series requires the primary succinamide.

Several compounds with modifications to the succinate region of the molecule were then prepared (Table 6). These included truncation of each group on the disubstituted succinate in order to explore the hydrophobic requirements of the binding site and to probe the active conformation. Deletion of either substituent of the succinate resulted in complete loss of activity. *Anti*-disubstituted succinates are known to adopt an extended, staggered conformation,[29] and this architecture appeared to be required. This interpretation was supported

Table 5. Selected secondary succinyl amides

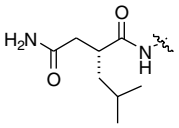
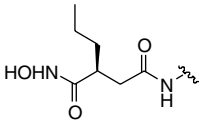
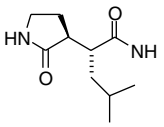
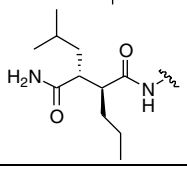


31-34

Compound	Substituent (R group)	IC_{50}[a] (nM)
31	*n*-Butyl	5000
32	2-Furylmethyl	3000
33	Cyclopentyl	6000
34	4-Phenyl-2-butenyl	3000

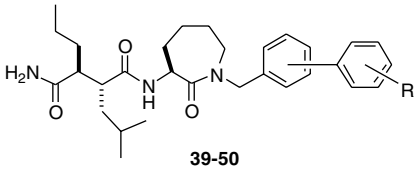
[a] Activity assayed in CHO N9 cells according to Ref. 16.

Table 6. Succinate modifications

Compound	Substituent (R group, structure xx)	IC_{50}[a] (nM)
35		>10,000
36		>10,000
37		>10,000
38		226

[a] Activity assayed in HEK 293 cells according to Ref. 16.

Table 7. Activity of selected biaryl succinyl amides



39-50

Compound	Biaryl	Substituent (R group, structure **10**)	IC_{50}[a] (nM)
39	3	2,4-Dichloro	14
40	3	4-Fluoro	191
41	3	4-Methyl	40
42	3	4-Methoxy	8
43 (RF978)	3	4-Fluoro, 3-chloro	17
44	3	4-Trifluoromethyl	10
45	4	4-Trifluoromethyl	90
46	3	3-Methoxy	150
47	3	3-Fluoro	300
48	3	2-Methoxy	30
49	3	2-Naphthyl	18
50	3	2-Tetrazolyl	1800
51	3	C(O)phenyl	100

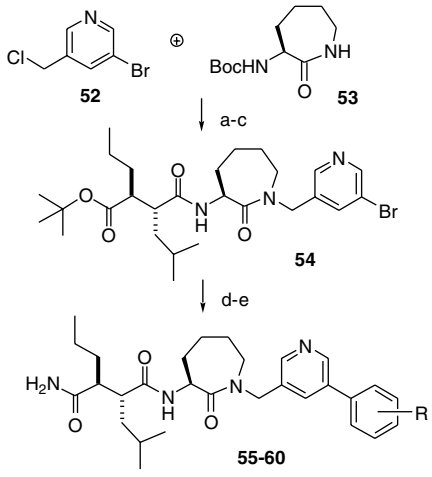
[a] Activity assayed in HEK 293 cells according to Ref. 16.

by the activity of compound **38**, which had both stereocenters inverted but still maintained good potency. Pyrrolidinone **37** was prepared by constraining the propyl side chain onto the primary amide, but this modification also proved inactive.[30]

With requirements for the *trans*-disubstituted succinyl primary amide established, the SAR of this series was explored using solid-phase chemistry similar to that used in the hydroxamate series, but linking through PAL resin (in analogy to Scheme 2). Substitution at the 4-position was also examined in this series by using the caprolactam prepared from 4-bromobenzyl bromide. Loading levels of the support-bound caprolactam halides were determined by cleavage and were 80–90% of theory. Overall yields from this procedure ranged from 5% to 15%, as the amount of cleavage from the resin during the Suzuki coupling step was quite high under the conditions we employed. Nevertheless, each compound was obtained in high purity.

As capropactam *N*-substituents were varied, the increased potency of the amide series was maintained relative to the hydroxamate series (Table 7). A limited comparison (compounds **44** and **45**) suggests that the 3-substituted biaryls are more potent than the 4-substituted biaryls. In the 3-substituted series, small lipophilic substituents on the 4-position tended to be more potent, as evidenced by the 4-methoxy and 4-trifluoromethyl compounds **42** and **44** (~10 nM activity). An attempt to install masked polarity in this position with the 2-tetrazole group resulted in a dramatic loss in affinity. Unfortunately, the simple 4-substituted derivatives including the potent derivatives **42** and **44** were poorly soluble, and we observed precipitation of compound from DMSO stocks upon long-term storage. The 4-fluoro-3-chloro derivative **43** (RF978) provided the best compromise between potency and maintained solubility in DMSO, although the aqueous solubility of this analog is also very poor (<1 μg/mL, see Table 10).

In an attempt to increase the solubility of this series, related compounds were prepared utilizing a 3-pyridyl biaryl group (Scheme 3). Alkylation of Boc-caprolactam using NaHMDS with the free base of 3-bromo-5-(chloromethyl)pyridine[31] proceeded smoothly (Scheme 3). The palladium-mediated coupling reaction was carried out at the stage of compound **54**, as all attempts to couple aryl halides with the succinyl primary amide already installed were unsuccessful. As is shown in Table 8, activity in the pyridyl series was roughly equivalent to activity in the biaryl series, although the 4-fluoro-3-chlo-



Scheme 3. Reagents and conditions: (a) 53, NaHMDS, −78 to 0 °C, then 52; (b) 50% TFA/DCM, rt; (c) 7, HATU, NMM, DMF, 50 °C, 48 h; (d) boronic acid, $Pd(PPh_3)_4$, 9:1 toluene/MeOH, 2 M Na_2CO_3, reflux, 16 h; (e) 50% TFA/DCM, rt; (f) HATU, NMM, NH_3; (g), DMF, rt.

Table 8. Activity of pyridyl biaryl succinyl amides

Compound	Biaryl	Substituent (R group, structure **10**)	IC_{50}[a] (nM)
55	3	4-Trifluoro methyl	17
56	3	4-Methoxy	32
57	3	4-Fluoro, 3-chloro	4
58	3	2-Pyridyl	8800
59	3	3-Pyridyl	2200

[a] Activity assayed in HEK 293 cells according to Ref. 16.

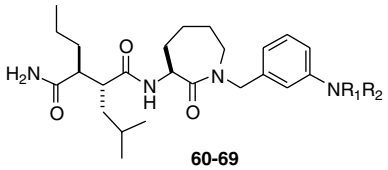
ro derivative **57** appeared slightly more potent than the best biaryls with an $IC_{50} = 4$ nM. These compounds, however, remained poorly soluble.[27]

Further improvements in solubility of the biaryl series were difficult due to the lack of tolerance for polarity at the distal end of the caprolactam substituent binding pocket. In the linker position, however, synthesis of the biaryl amine corresponding to the biaryl ether **30** through Buchwald/Hartwig amination[28] of the iodophenyl intermediate provided compound **60**, which served as the lead for a series of aryl amines (Table 9).

In this series, 3-substitution was preferred over 4-substitution, however none of the substituted derivatives proved more potent than the parent aniline **60**. A variety of non-aryl amines were also synthesized, with piperidine **69** showing modest activity (74 nM). Other non-aryl derivatives, or amines with more polar substituents, were prepared but were less potent than compound **69** (data not shown).

A preliminary in vivo profile of compounds **30**, **43**, and **60** was collected in the beagle dog. Compounds were dosed in cassette format with up to nine other compounds. Doses were kept low (0.25 mg/kg iv and 0.5 or 1.0 mg/kg po) to help minimize potential compound interactions. Pharmacokinetic parameters for the compounds are presented in Table 10. In the dog, compound **30** had low oral bioavailability, poor overall exposure,

Table 9. Biarylamines



60-69

Compound	Substituent (NR_1, R_2)	IC_{50}[a] (nM)
60	NHphenyl	15
61	NH(2-chlorophenyl)	29
62	NH(3-methylphenyl)	17
63	NH(3-methoxyphenyl)	54
64	NH(3,4-methylenedioxyphenyl)	152
65	NH(4-fluoro, 3-chlorophenyl)	17
66	NH(4-methoxyphenyl)	160
67	NH(4-trifluoromethylphenyl)	30
68	NH(3,4-methylenedioxyphenyl)	150
69	1-Piperidyl	74

[a] Activity assayed in CHO N9 cells according to Ref. 16.

Table 10. Pharmacokinetics of compounds **30**, **43**, and **60**

Parameter	Compound **30** po at 0.5 mg/kg	Compound **43** po at 1.0-mg/kg	Compound **60** po at 0.5 mg/kg
Solubility pH 7.4 (mg/mL)	0.001	0.0001	n.d.
Fu[a] (%, dog)	0.4	bql	1.5
CI (l/h/kg)	1.9	0.4	0.6
V_{ss} (l/kg)	1.3	0.8	0.4/1.1[c]
$t_{1/2}$ (h)	0.5	1.3	1.0
C_{max} (nM)	25	116	17/73[c]
F[b] (%)	8	25	3/21[c]

[a] bql, below quantifiable limit of assay.
[b] Fraction unbound bioavailability.
[c] Two numbers represent data from two different animals.

and was rapidly cleared. Compound **43** had an improved profile, with substantially lower clearance and moderate bioavailability and half-life leading to somewhat higher overall exposure. Compound **60** had a high level of variability between the two animals, but had a moderate to poor overall exposure. Taken together, these data suggested that further improvements to the caprolactam succinamide series were needed.

In addition to cleavage of APP, it has been shown that γ-secretase also functions to cleave the Notch receptor, and inhibition of γ-secretase leads to defects in Notch function.[32–34] In adult animals, disruption of Notch signaling through γ-secretase blockade has been shown to cause intestinal goblet cell hyperplasia, a process that leads to the formation of intestinal lesions.[35–38] Thus, the development of γ-secretase inhibitors as therapeutic agents will likely require selectivity for inhibition of brain Aβ production versus peripheral Notch function. These three representative compounds were therefore profiled for their ability to block γ-secretase-mediated Notch signaling. As is shown in Table 11, all three compounds are inhibitors of Notch signaling with cellular potencies similar to their potency as inhibitors of Aβ production.

In summary, a series of succinoyl-caprolactam γ-secretase inhibitors was synthesized to follow the lead structure SR973 (**1**), which was identified from a screening campaign. Optimization of the series using efficient parallel synthesis led to the identification of inhibitors with <10 nM cellular potency for Aβ production inhibition. This series also provided reagents which enabled examination of aspects of γ-secretase biology. Compound **51** ($IC_{50} = 100$ nM) with a 3-linked benzophenone substituent was designed as a potential photoaffinity probe. Reduction of the related allyl precursor with tritium provided a ligand that was used to identify presenilins 1 and 2 as the molecular targets of these small-molecule

Table 11. Notch inhibition of compounds **30**, **43**, and **60**

Parameter	Compound **30**	Compound **43**	Compound **60**
Aβ IC_{50} (nM)	50	17	15
Notch IC_{50} (nM)	82 ± 22	18 ± 15	64 ± 33

Activity assayed in murine 3T3 cells using murine Notch ΔE (see Ref. 42 for full experimental details).

Aβ production inhibitors. Similarly, reduction of an allyl precursor of amide **30** (RE987) provided a radioligand which enabled the construction of a binding assay which has been used to examine the potency of presenilin inhibitors under cell-free conditions.[29] These results and the SAR described in this work provided the groundwork for the production of other series of γ-secretase inhibitors which will be reported in due course.

References and notes

1. Selkoe, D. J. *Ann. Intern. Med.* **2004**, *140*, 627.
2. Plosker, G. L.; Keating, G. M. *Dis. Manage. Health Outcomes* **2004**, *12*, 55.
3. Kornilova, A. Y.; Wolfe, M. S. *Annu. Rep. Med. Chem.* **2003**, *38*, 41.
4. Josien, H. *Curr. Opin. Drug Discov. Dev.* **2002**, *5*, 513.
5. John, V.; Beck, J. P.; Bienkowski, M. J.; Sinha, S.; Heinrikson, R. L. *J. Med. Chem.* **2003**, *46*, 4625.
6. Lin, X.; Koelsch, G.; Wu, S.; Downs, D.; Dashti, A.; Tang, J. *Proc. Natl. Acad Sci. U.S.A.* **2000**, *97*, 1456.
7. Hussain, I.; Powell, D.; Howlett, D. R.; Tew, D. G.; Meek, T. D.; Chapman, C.; Gloger, I. S.; Murphy, K. E.; Southan, C. D.; Ryan, D. M.; Smith, T. S.; Simmons, D. L.; Walsh, F. S.; Dingwall, C.; Christie, G. *Mol. Cell Neurosci.* **1999**, *14*, 419.
8. Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashier, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Heinrikson, R. L.; Gurney, M. E. *Nature* **1999**, *402*, 533.
9. Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S. M.; Wang, S.; Walker, D.; John, V. *Nature* **1999**, *402*, 537.
10. Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. *Science* **1999**, *286*, 735.
11. Kimberly, W. T.; LaVoie, M. J.; Ostaszewski, B. L.; Ye, W.; Wolfe, M. S.; Selkoe, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6382.
12. Fraering, P. C.; Ye, W.; Strub, J.-M.; Dolios, G.; LaVoie, M. J.; Ostaszewski, B. L.; van Dorsselaer, A.; Wang, R.; Selkoe, D. J.; Wolfe, M. S. *Biochemistry* **2004**, *43*, 9774.
13. De Strooper, B. *Neuron* **2003**, *38*, 9.
14. Lanz, T. A.; Himes, C. S.; Pallante, G.; Adams, L.; Yamazaki, S.; Amore, B.; Merchant, K. M. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 864.
15. Dovey, H. F.; John, V.; Anderson, J. P.; Chen, L. Z.; De Saint Andrieu, P.; Fang, L. Y.; Freedman, S. B.; Folmer, B.; Goldbach, E.; Holsztynska, E. J.; Hu, K. L.; Johnson-Wood, K. L.; Kennedy, S. L.; Kholodenko, D.; Knops, J. E.; Latimer, L. H.; Lee, M.; Liao, Z.; Lieberburg, I. M.; Motter, R. N.; Mutter, L. C.; Nietz, J.; Quinn, K. P.; Sacchi, K. L.; Seubert, P. A.; Shopp, G. M.; Thorsett, E. D.; Tung, J. S.; Wu, J.; Yang, S.; Yin, C. T.; Schenk, D. B.; May, P. C.; Altstiel, L. D.; Bender, M. H.; Boggs, L. N.; Britton, T. C.; Clemens, J. C.; Czilli, D. L.; Dieckman-McGinty, D. K.; Droste, J. J.; Fuson, K. S.; Gitter, B. D.; Hyslop, P. A.; Johnstone, E. M.; Li, W. Y.; Little, S. P.; Mabry, T. E.; Miller, F. D.; Ni, B.; Nissen, J. S.; Porter, W. J.; Potts, B. D.; Reel, J. K.; Stephenson, D.; Su, Y.; Shipley, L. A.; Whitesitt, C. A.; Yin, T.; Audia, J. E. *J. Neurochem.* **2001**, *76*, 173.
16. Higaki, J. N.; Chakravarty, S.; Bryant, C. M.; Cowart, L. R.; Harden, P.; Scardina, J. M.; Mavunkel, B.; Luedtke, G. R.; Cordell, B. *J. Med. Chem.* **1999**, *42*, 3889.
17. Xue, C.-B.; Voss, M. E.; Nelson, D. J.; Duan, J. J. W.; Cherney, R. J.; Jacobson, I. C.; He, X.; Roderick, J.; Chen, L.; Corbett, R. L.; Wang, L.; Meyer, D. T.; Kennedy, K.; DeGrado, W. F.; Hardman, K. D.; Teleha, C. A.; Jaffee, B. D.; Liu, R.-Q.; Copeland, R. A.; Covington, M. B.; Christ, D. D.; Trzaskos, J. M.; Newton, R. C.; Magolda, R. L.; Wexler, R. R.; Decicco, C. P. *J. Med. Chem.* **2001**, *44*, 2636.
18. Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 111.
19. Xue, C.-B.; He, X.; Roderick, J.; Corbett, R. L.; Duan, J. J. W.; Liu, R.-Q.; Covington, M. B.; Qian, M.; Ribadeneira, M. D.; Vaddi, K.; Christ, D. D.; Newton, R. C.; Trzaskos, J. M.; Magolda, R. L.; Wexler, R. R.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4299.
20. Xue, C.-B.; He, X.; Roderick, J.; Corbett, R. L.; Duan, J. J. W.; Liu, R.-Q.; Covington, M. B.; Newton, R. C.; Trzaskos, J. M.; Magolda, R. L.; Wexler, R. R.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *73*, 4293.
21. Duan, J. J. W.; Chen, L.; Lu, Z.; Wasserman, Z. R.; Maduskuie, T. P.; Liu, R.-Q.; Covington, M. B.; Vaddi, K. G.; Qian, M.; Voss, M. E.; Xue, C.-B.; Hardman, K. D.; Ribadeneira, M. D.; Newton, R. C; Magolda, R. L.; Christ, D. D.; Decicco, C. P. *Abstracts of Papers*, 224th ACS National Meeting, Boston, MA, United States, Aug 18–22, 2002; MEDI-426.
22. Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
23. Wolfe, M. S.; Kopan, R. *Science* **2004**, *305*, 1119.
24. Wolfe, M. S.; Angeles, J. D. L.; Miller, D. D.; Xia, W.; Selkoe, D. *J. Biochem.* **1999**, *38*, 1223.
25. Wolfe, M. S.; Xia, W.; Ostaszewski, B. L.; Diehl, T. S.; Kimberly, W. T.; Selkoe, D. J. *Nature* **1999**, *398*, 513.
26. Wolfe, M. S.; Xia, W.; Moore, C. L.; Leatherwood, D. D.; Ostaszewski, B.; Rahmati, T.; Donkor, I. O.; Selkoe, D. J. *Biochemistry* **1999**, *38*, 4720.
27. Steiner, H.; Kostka, M.; Ronig, H.; Basset, G.; Pesold, B.; Hardy, J.; Capell, A.; Meyn, L.; Grim, M. L.; Baumeister, R.; Fechteler, K.; Haass, C. *Nat. Cell Biol.* **2000**, *2*, 848.
28. Alsina, J.; Yokum, T. S.; Albericio, F.; Barany, G. *J. Org. Chem.* **1999**, *64*, 8161.
29. Xue, C.-B.; He, X.; Roderick, J.; Degrado, W. F.; Cherney, R. J.; Hardman, K. D.; Nelson, D. J.; Copeland, R. A.; Jaffee, B. D.; Decicco, C. P. *J. Med. Chem.* **1998**, *41*, 1145.
30. This compound was prepared by ozonolysis of the allyl sidechain, followed by reduction of the resultant cyclic hemiaminal with TFA/Et_3SiH.
31. Nutaitis, C. F.; Ledeboer, M. W. *Org. Prep. Proc. Int.* **1992**, *24*, 143.
32. De Strooper, B.; Annaert, W.; Cupers, P.; Saftig, P.; Craessaerts, K.; Mumm, J. S.; Schroeter, E. H.; Schrijvers, V.; Wolfe, M. S.; Ray, W. J.; Goate, A.; Kopan, R. *Nature* **1999**, *398*, 518.
33. Ye, Y.; Lukinova, N.; Fortini, M. E. *Nature* **1999**, *398*, 525.
34. Struhl, G.; Greenwald, I. *Nature* **1999**, *398*, 522.
35. vanEs, J. H.; vanGijn, M. E.; Riccio, O.; van den Born, M.; Vooijs, M.; Begthel, H.; Cozijnsen, M.; Robine, S.; Winton, D. J.; Radtke, F.; Clevers, H. *Nature* **2005**, *435*, 959.
36. 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. *J. Biol. Chem.* **2003**, *278*, 46107.
37. Milano, J.; McKay, J.; Dagenais, C.; Foster-Brown, L.; Pognan, F.; Gadient, R.; Jacobs, R. T.; Zacco, A.; Greenberg, B.; Ciaccio, P. J. *Toxicol. Sci.* **2004**, *82*, 341.
38. 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. J.; Zhang, L.; Higgins, G. A.; Parker, E. M. *J. Biol. Chem.* **2004**, *279*, 12876.
39. Schroeter, E. H.; Kisslinger, J. A.; Kopan, R. *Nature* **1998**, *393*, 382.
40. Kopan, R.; Schroeter, E. H.; Weintraub, H.; Nye, J. S. *Proc. Natl. Acad Sci. U.S.A.* **1996**, *93*, 1683.
41. All new compounds reported herein gave satisfactory analytical data including parent mass, proton NMR spectral integrity, and high purity by HPLC characterization. Compounds characterized in vivo also gave satisfactory combustion analysis data.
42. Notch signaling assay: NIH3T3 cells were maintained in growth media (Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL) containing 10% calf serum (Gibco-BRL), Penicillin/Streptomycin (Gibco-BRL), 2 mM L-glutamine (Gibco-BRL), and 1 mM pyruvate (Cellgro)) and were treated with compounds in defined media (DMEM, 2mM L-glutamine, penicillin/streptomycin, 1 mM pyruvate, and 0.0125% BSA (Sigma)). The mouse Notch-1 AE construct was generated essentially as described.[39,40] The CBFl-luciferase reporter construct consists of four tandem repeats of the CBF1 binding element in the pGL3-Promoter vector (Promega). NIH 3T3 cells were transiently transfected using Effectere (Qiagen) according to the manufacturer's directions. Cells were transfected in batch with a combination of Notch ΔE, vector DNA, and the CBFl-luciferase reporter construct for 24 h. Following transfection, cells were replated in 96-well plates in the presence of defined media and treated with compound serial dilutions for 20 h. Luciferase activity was measured using Luc Screen (Tropix) according to the manufacturer's directions. Dose–response curves were analyzed using GraphPad Prism (GraphPad Software) and nonlinear regression analysis.