Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5790–5795
Bioorganic & Medicinal Chemistry Letters

Amino-caprolactam derivatives as γ-secretase inhibitors

Michael F. Parker,[a,*] Joanne J. Bronson,[a] Donna M. Barten,[b] Jason A. Corsa,[b] Wengsheng Du,[b] Kevin M. Felsenstein,[b,†] Valerie L. Guss,[b] Darcy Izzarelli,[b] Alice Loo,[b] Kate E. McElhone,[a,‡] Larry R. Marcin,[a] Ramesh Padmanabha,[b] Roger Pak,[b] Craig T. Polson,[b] Jeremy H. Toyn,[b] Sam Varma,[b] Jian Wang,[c] Victoria Wong,[b] Ming Zheng[d] and Susan B. Roberts[b]

[a]*Department of Discovery Chemistry, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA*
[b]*Department of Neuroscience Biology, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA*
[c]*Department of Bioanalytical Research, Bristol-Myers Squibb Research and Development, Rt. 206, Lawrenceville, NJ 08543, USA*
[d]*Department of Drug Metabolism and Pharmacokinetics, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA*

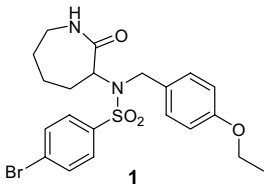
Received 24 July 2007; revised 20 August 2007; accepted 23 August 2007
Available online 26 August 2007

Abstract—A series of amino-caprolactam sulfonamides were developed from a screening hit. Compounds with good in vitro and in vivo γ-secretase activity are reported.

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder characterized by memory impairment and cognitive dysfunction.[1] AD is characterized pathologically by the accumulation of senile (neuritic) plaques, neurofibrillary tangles, amyloid deposition in neural tissues and vessels, synaptic loss, and neuronal death.[2a,b] AD is the most common form of dementia and now represents the third leading cause of death after cardiovascular disorders and cancer.[3] Evidence suggests the accumulation of β-amyloid peptides (Aβ) is responsible for the neuronal toxicity that is associated with AD.[4] Aβ peptides are generated by sequential proteolytic cleavage of a 695–770 amino acid precursor protein (APP)[5] by the action of β- and γ-secretases.

We have been interested in identification of compounds that inhibit the production of β-amyloid peptide (β-AP) from β-amyloid precursor protein (β-APP), since such agents may be useful for the treatment or prevention of Alzheimer's disease. A cell-based assay measuring Aβ production[6] from membranes isolated from cells expressing β-APP[7] was performed on our internal compound collection and the caprolactam sulfonamide derivative **1**[8,9] (Fig. 1) was found to be a promising inhibitor of γ-secretase (IC_{50} = 120 nM). Herein we report the SAR within this series of caprolactam γ-secretase inhibitors.

Starting from commercially available D- or L-lysine **2**, amino caprolactam **3** was conveniently made by ring closure of the trimethylsilyl amino ester[10] as shown in



1

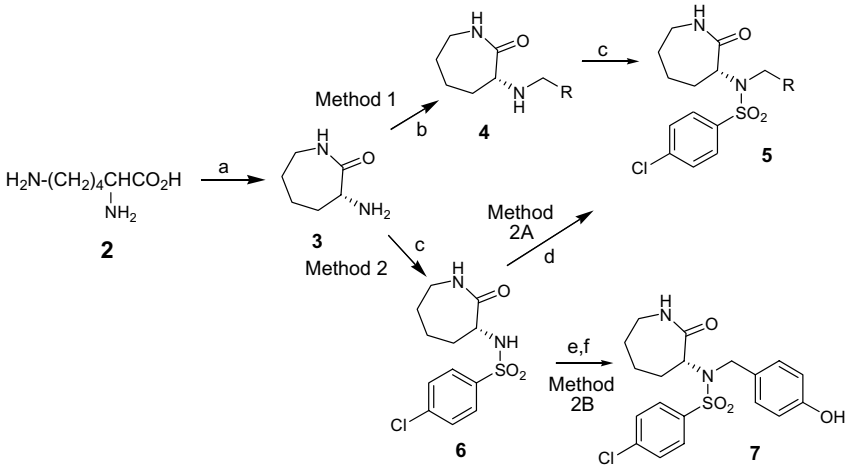
Figure 1.

Keywords: γ-Secretase inhibitors; Alzheimer's disease; *N*-Benzyl amino caprolactams.
* Corresponding author. Tel.: +1 203 677 6466; fax: +1 203 677 7702; e-mail: parkerm@BMS.com
† Present address: Johnson & Johnson PRD, Spring House, PA 19477, USA.
‡ Present address: Johnson & Johnson Pharmaceutical Research & Development, La Jolla, CA 92121, USA.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.08.047

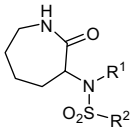
Scheme 1. This synthetically useful chiral amine underwent reductive amination by treatment with an aldehyde and sodium cyanoborohydride in methanol to provide intermediate **4**. The resulting secondary amines were treated with a variety of sulfonyl chlorides to produce the desired analog **5** shown in Scheme 1. Alternatively the chiral amino caprolactam **3** could first be sulfonylated to afford intermediate **6**. The resulting secondary sulfonamides could be preferentially alkylated with an array of alkyl or benzyl halides in DMF with K_2CO_3 (method 2A). In the preparation of the 4-hydroxybenzyl benzenesulfonamide analog **7**, synthesis via method 1 or method 2A was unsuccessful because of decomposition of the benzylic halide under the usual reaction conditions. The alkylation of the sulfonamide was accomplished instead by a standard Mitsunobu reaction on the sulfonamide caprolactam **6** with the differentially protected ether alcohol, 4-(*tert*-butyldimethylsilyloxy) benzyl alcohol. The crude product of this reaction was then deprotected with tetrabutyl ammonium fluoride in THF, to yield the desired phenol **7**.

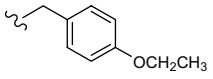
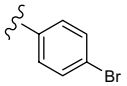
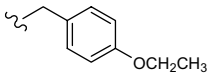
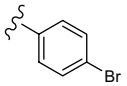
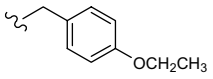
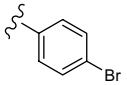
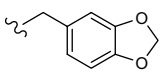
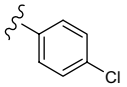
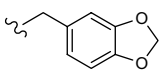
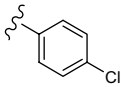
Optimization of the 4-bromosulfonamide caprolactam screening lead first focused on determination of whether the enantiomers had differential potency. Comparison of the enantiomers **8** and **9** clearly showed the R-isomer, which was derived from D-lysine, to be significantly, and as with **9**, exceptionally more potent.[12] Likewise, with the enantiomer pair **10** and **11**, the R-isomer **11** was >300× more potent (Table 1).



Scheme 1. General synthesis of *N*-benzyl-*N*-benzenesulfonamide derivatives. Reagents: (a) hexamethyldisilazane, chlorotrimethylsilane, xylene, 95%; (b) alkyl aldehyde or benzaldehyde, $ZnCl_2$, $NaCNBH_4$, MeOH, 40–80%; (c) 4-chlorobenzenesulfonyl chloride, Et_3N, CH_2Cl_2, 92%; (d) alkyl or benzyl halide, K_2CO_3, DMF, 30–88%; (e) triphenylphosphine, 4-(*tert*-butyldimethylsilyloxy) benzyl alcohol, diethylazodicarboxylate, THF, 58%; (f) TBAF, THF, 72%.

Table 1. Inhibitory activities of caprolactam enantiomers



Ex. #	R^1	R^2	Method	Isomer	Aβ40 IC_{50} (nM)[a]
1			2A	D/L	120
8			2A	S	3400
9			2A	R	13
10			1	S	3200
11			1	R	9.7

[a] Values are means of four experiments, with 12 drug concentrations in each exp.; intra-assay variance <10%.[6]

We next probed the effect of the lactam ring size[11] on potency. As exemplified by compounds **12**, **13**, and **14** in Figure 2, both the 5- and 6-membered lactams were significantly less potent than the corresponding over the 7-membered ring **14**. As a result of these findings, subsequent analogs were focused on the caprolactam core (Fig. 2).

Modification of the aryl moiety attached to the sulfonamide sulfur atom was also undertaken. As shown in Table 2, the 4-chlorobenzenesulfonamide (e.g., **16** and **21**) was optimal for potency. Introduction of a heteroaryl sulfonamide, as in the thiophene analog **17**, led to a 50-fold loss in potency. Substitution of the 4-chloro by fluorine (e.g., **15** and **20**) or removal of the chlorine as in **19** resulted in a modest decrease in potency. Substitution with an alkyl group as in **18** was quite detrimental to potency.

Our strategy was then to examine the nature of the substituents at R^1 on the sulfonamide nitrogen while keeping 4-chlorophenylsulfonamide constant. Compounds shown in Table 3 result from examination of various benzylic substituents, while compounds in Table 4 illustrate a variety of non-benzylic groups. As shown in Table 3, *para*-substitution on the benzyl side chain generally improved potency when compared to the unsubstituted ring system **22**. Strongly electron-donating *para*-substituents conferred the greatest potency, as seen with the methoxyphenyl analog **23** (IC_{50} = 2.8 nM) and the phenol **7** (IC_{50} = 5.0 nM). While electron-rich systems were generally more potent (e.g., aniline **25**,

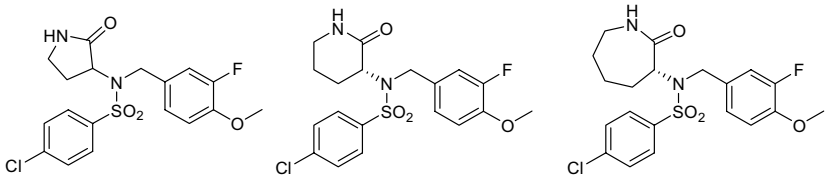
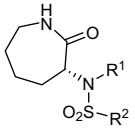
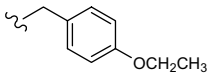
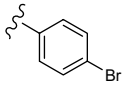
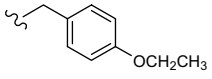
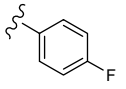
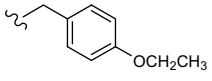
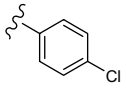
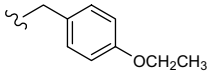
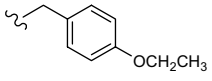
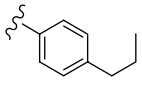
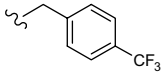
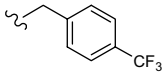
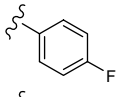
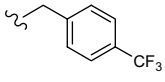
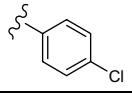


Figure 2.

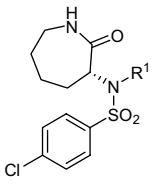
Table 2. Modification of the aryl sulfonamide moiety



Ex. #	R^1	R^2	Method	Aβ40 (IC_{50} nM)[a]
9			2A	13
15			1	57
16			2A	9.0
17			1	830
18			1	7700
19			1	180
20			1	25
21			1	21

[a] Values are means of four experiments, with 12 drug concentrations in each exp.; intra-assay variance <10%.

Table 3. Inhibitory activities of R^1 benzylic substituents



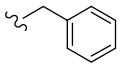
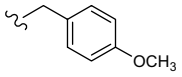
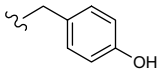
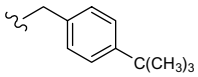
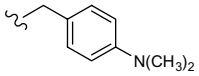
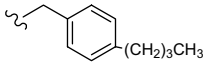
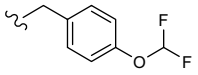
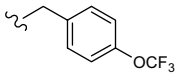
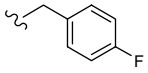
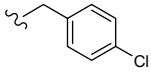
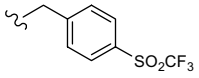
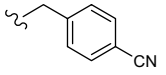
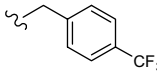
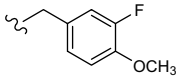
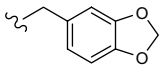
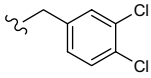
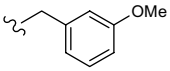
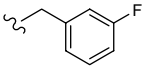
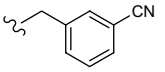
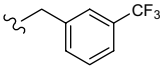
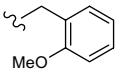
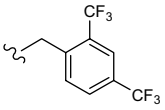
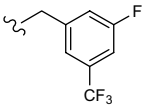
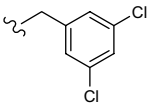
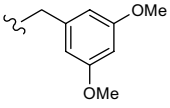
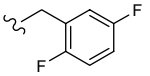
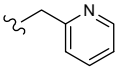
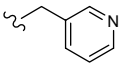
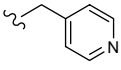
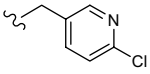
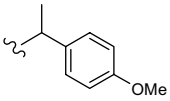
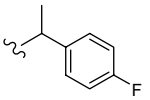
Ex. #	R^1	Method	Aβ40 (IC_{50} nM)[a]
22		1	65
23		1	2.8
7		2B	5.0
24		2A	13
25		1	11
26		2A	76
27		2A	18
28		2A	45
29		2A	33
30		2A	12
31		2A	25
32		2A	41
21		1	21
14		1	9.1
11		1	9.7
33		2A	15

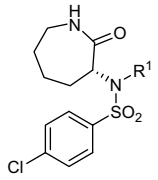
Table 3 (*continued*)

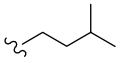
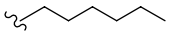
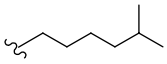
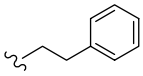
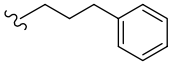
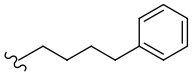
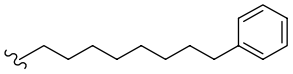
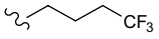
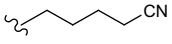
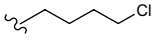
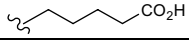
Ex. #	R^1	Method	Aβ40 (IC_{50} nM)[a]
34		2A	93
35		2A	29
36		2A	64
37		2A	60
38		2A	820
39		2A	660
40		2A	130
41		2A	550
42		2A	130
43		2A	67
44		1	460
45		1	120
46		1	340
47		1	27
48		2B	690
49		2B	4500

[a] Values are means of four experiments, with 12 drug concentrations in each exp.; intra-assay variance <10%.

IC_{50} = 11 nM; *tert*-butylphenyl **24**, IC_{50} = 12 nM), electron-withdrawing groups such as CF_3SO_2 (**28**, IC_{50} = 25 nM) and CN (**32**, IC_{50} = 41 nM) were also tolerated. Combination of a *para*-OR group with a *meta*-substituent was also acceptable as seen with the 3-fluoro-4-methoxy phenyl analog **14** (IC_{50} = 9.1 nM) and the methylenedioxyphenyl derivative **11** (IC_{50} = 9.7 nM). By comparison, single *meta*-substitution of the benzylic group was generally less favored than *para*-substitution as illustrated by comparison of **23/34**, **32/36**, and **21/37**. *Ortho*-substitution was significantly disfavored as seen with compounds **38** and **39**. Disubstituted benzyl groups lacking *para*-substituents (e.g., **40**–**43**) were also less potent.

Table 4. Inhibitory activities of R^1 alkyl substituents



Ex. #	R^1	Method	Aβ40 (IC_{50} nM)[a]
50		2A	1400
51		1	620
52		2A	110
53		2A	85
54		2A	1100
55		2A	67
56		2A	470
57		2A	1000
58		2A	330
59		2A	180
60		2B	150
61	 *b	2B	3900

[a] Values are means of four experiments, with 12 drug concentrations in each exp.; intra-assay variance <10%.
[b] Synthesized from the bromo ester, followed by saponification to yield the acid **61**.

In order to improve solubility, we examined a series of pyridylmethyl side chains. The 3-pyridyl analog **45** was more potent than the 2-pyridyl **44** and the 4-pyridyl **46** isomers. Attenuating the p*K*a of the pyridyl ring by adding a 4-chloro substitution as in **47** had a positive effect on binding. With the exception of **47**, the pyridyl series was significantly less potent than the simple methoxy- or methylenedioxy phenyl compounds. Finally, branching at the benzylic position was examined. Addition of an α-methyl group, as shown in examples **48** and **49**, resulted in a significant loss of potency.

Analogs with alkyl side chains on the sulfonamide nitrogen were also examined (Table 4). Elimination of the aryl ring had a negative impact on the potency in this series. Interestingly, we found that substantial length was required to impart even modest activity in the simple alkyl series, as shown with compounds **50**–**53**. In the case of the phenyl alkyl derivatives, extending chain length also improved potency, with the phenylpropyl derivative **55** being the most potent in the series. However, further extension of the phenyl derivative chain length led to a decrease in potency. Capping the terminus of the alkyl side chain with functional groups such as CF_3, CN, Cl or CO_2H (**58**–**61**, respectively) did not improve potency to the level of benzylic substituents, although the trifluoromethylpropyl derivative **60** had better potency than the simple propyl derivative **50**. Even the best compound in the alkyl series was still 10-fold less active than in the benzyl series.

To further assess the caprolactam series, the methoxyphenyl (**23**) and the dimethylaniline (**25**) were evaluated in vivo[13] for the reduction of brain concentrations of Aβ. The compounds were selected for further study based on their potency in the Aβ40 in vitro assay.[6] Transgenic mice (Tg2576 mice,[14] 3–6 months of age) were treated by oral gavage with test compounds at a dose of 200 μmol/kg. The effects of **23** and **25** on Aβ in brain and plasma concentrations and relative compound concentrations at 3 h post dose are shown in Table 5. Both compounds **23** and **25** are highly protein bound (>97%) but were found to diminish brain and plasma concentrations of Aβ as measured by a standard ELISA for Aβ40.[6] Compound concentrations were analyzed by LC–MS–MS method. Although **23** is ~4-

Table 5. In vivo efficacy of potent caprolactam sulfonamides

Ex. #	Aβ40 IC_{50} (nM)	Aβ42 IC_{50} (nM)	% inhibition brain[13] (concn)	% inhibition plasma[13] (concn)	B/P ratio
23	2.8	2.4	49% (2200 nM)	47% (280 nM)	7
25	11	7.8	52% (4200 nM)	61% (1600 nM)	2.8

fold more potent than the aniline **25**, **25** has higher relative brain and plasma exposures. This may explain why the in vivo potencies of these compounds are comparable.

In summary, potent γ-secretase inhibitors emerged from SAR studies and optimization of an initial amino caprolactam screening hit. The most potent compounds to emerge from this study were the 4-methoxy benzyl compound **23** and the *N*,*N*-dimethyl analog **25**. These compounds were approximately 50-fold more potent than the original screening hit and markedly reduced the concentration of Aβ in the brain and plasma in transgenic Tg2576 mice. However, **23** and **25** were also found to be nanomolar inhibitors of the human CYP450 isoforms 3A4 and 2C19. Further investigations are focused on the removal of these liabilities from this and similar sulfonamide series.

References and notes

1. Scheff, S. W.; Price, D. A.; Schmitt, F. A.; Mufson, E. J. *Neurobiol. Aging* **2006**, *27*, 1372.
2. (a) Clark, C. M.; Karlawish, J. H. *Ann. Intern. Med.* **2003**, *138*, 400; (b) Hardy, J.; Higgins, G. A. *Science* **1992**, *256*, 184.
3. Nguyen, J.; Yamani, A.; Kiso, Y. *Curr. Pharm. Des.* **2006**, *12*, 4295.
4. Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, S.; Rowan, M. J.; Delkow, D. J. *Nature* **2002**, *416*, 535.
5. Selkow, D. J.; Podlisny, M. B.; Joachim, C. l.; Vickers, E. A.; Lee, G.; Fritz, L. C.; ltersdorf, T. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7341.
6. H4 cells stably transfected with APP751 containing the Swedish mutation (H4 APP751 SWE clone 8.20, developed at BMS) were maintained in log phase through twice weekly passage at a 1:20 split. For IC_{50} determinations, 1.5×10^4 cells/well in DMEM containing 0.0125% BSA (Sigma A8412) were plated directly into 384-well compound plates (Costar 3709) containing 0.1 μl serially diluted compound in DMSO. Following incubation for 19 h in 5% CO_2 at 37 °C, plates were briefly centrifuged (1000 rpm, 5 min). A 10 μl aliquot from each well was transferred to a second assay plate (Costar 3709) for Aβ1–40 measurements. Antibody cocktails were freshly prepared by dilution into 40 mM Tris–HCl (pH 7.4) with 0.2% BSA and added to assay plates. For the Aβ40 measurements, antibodies specific for the Aβ40 neoepitope (TSD, developed at BMS; conjugated to the Wallac reagent (Perkin-Elmer)) and 26D6 as described above were mixed and 20 μl of the mixture was added to the 10 μl aliquots which had been removed previously from the cell plate yielding a final concentration of 1.6 ng/well TSD and 17.5 ng/well 26D6. Assay plates containing antibodies were sealed with aluminum foil and incubated overnight at 4 °C. Signal was determined using a Viewlux counter (Perkin-Elmer) and IC_{50} values determined using curve fitting in CurveMaster (Excel-Fit based data analysis package). Also, see Smith, D. W.; Munoz, B.; Srinirvasan, K.; Bergstrom, C. P.; Chaturvedula, P. V.; Deshpande, M. S.; Keavy, D. J.; Lau, W. Y.; Parker, M. F.; Sloan, C. P.; Wallace, O. B.; Wang, H. H. WO 0050391.
7. Roberts, S. B.; Hendrick, J. P.; Vinitsky, A.; Lewis, M.; Smith, D. W.; Pak, R. Isolation of functionally active γ-secretase presenilin 1 complex and fluorescence assay for γ-secretase activity and inhibitors. US 2004, 0121411 A1.
8. Purchased from ComGenex Corporation as part of a collection of sulfonamides.
9. Recently disclosed in a patent, Martin, N.; preparation of *N*-substituted heterocyclic sulfonamides for treating cognitive disorders. US 2006/0270657A1.
10. Pellegata, R.; Pinza, G.; Pifferi, G. *Synthesis* **1978**, 614.
11. The 3 amino-2-piperidone was synthesized from D-ornithine following the method of Pellegata (9). The 3-amino-2-pyrrolidinone was purchased from Ramidus AB.
12. Possible clarifications for the 10-fold increase in activity include but are not limited to: antagonism or partial agonist activity by the inactive enantiomer, chiral ligand regulated enzyme activity, allosteric binding by one of the enantiomers or even relative error in the binding assay. These hypothetical explanations are being explored further.
13. Determination of Aβ, full length APP, and APP carboxy-terminal fragments, brain tissues was performed following the procedure as described previously. Barten, D. M.; Guss, V. L.; Corsa, J. A.; Loo, A.; Hansel, S. B.; Zheng, B.; Munoz, K.; Srinivasan, K.; Wang, B.; Roberson, B. J.; Polson, C. T.; Wang, J.; Roberts, S. B.; Hendrick, J. P.; Anderson, J. J.; Loy, J. K.; Denton, R.; Verdoorn, T. A.; Smith, D. W.; Felsenstein, K. M. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 635.
14. Hsiao, K.; Chapman, P.; Nilsen, S.; Eckman, C.; Harigaya, Y.; Younkin, S.; Yang, F.; Cole, G. *Science* **1996**, *274*, 99.