Bioorganic & Medicinal Chemistry Letters 19 (2009) 4920–4923

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis, and structure–activity relationship of novel orally efficacious pyrazole/sulfonamide based dihydroquinoline γ-secretase inhibitors

Anh P. Truong [a], Danielle L. Aubele [a], Gary D. Probst [a,*], Martin L. Neitzel [a], Chris M. Semko [a], Simeon Bowers [a], Darren Dressen [a], Roy K. Hom [a], Andrei W. Konradi [a], Hing L. Sham [a,b], Albert W. Garofalo [a], Pamela S. Keim [b], Jing Wu [b], Michael S. Dappen [b], Karina Wong [c], Erich Goldbach [c], Kevin P. Quinn [c], John-Michael Sauer [c], Elizabeth F. Brigham [d], William Wallace [e], Lan Nguyen [e], Susanna S. Hemphill [e], Michael P. Bova [e], Guriqbal Basi [e]

[a] Department of Medicinal Chemistry, Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, CA 94080, United States
[b] Department of Process and Analytical Chemistry, Elan Pharmaceuticals, 1000 Gateway Boulevard, South San Francisco, CA 94080, United States
[c] Department of Lead Finding, Drug Disposition, and Safety Evaluation, Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, CA 94080, United States
[d] Department of In Vivo Pharmacology, Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, CA 94080, United States
[e] Department of Biology, Elan Pharmaceuticals, 1000 Gateway Boulevard, South San Francisco, CA 94080, United States

ARTICLE INFO

Article history:
Received 22 June 2009
Accepted 20 July 2009
Available online 23 July 2009

Keywords:
γ-Secretase inhibitors
Alzheimer's disease

ABSTRACT

In this Letter, we report our strategy to design potent and metabolically stable γ-secretase inhibitors that are efficacious in reducing the cortical Aβx-40 levels in FVB mice via a single PO dose.

Alzheimer's disease (AD) is a form of senile dementia, characterized by a progressive loss of memory and cognitive ability, affecting 24 million elderly people worldwide.[1] The pathology of this neurodegenerative disorder uniquely manifests itself with the presence of extraneuronal aggregation of plaques composed of β-amyloid peptides (Aβ).[2] Aβ-peptides are derived from the sequential proteolytic cleavage of the β-amyloid precursor protein (β-APP) by two aspartic acid proteases, referred to as β- and γ-secretase, respectively.[3] Inhibition of either protease has been demonstrated to result in reduction of brain Aβ-peptide in preclinical studies.[4] Inhibitors of either protease offer attractive candidates as disease-modifying treatments for people afflicted with this debilitating malady.[5]

Tetrahydroquinoline sulfonamides have been previously reported as γ-secretase inhibitors.[6] Herein, we will describe the properties of our pyrazole/sulfonamide based dihydroquinoline series of γ-secretase inhibitors[7] that can be divided into four components: the aryl, sulfonamide, pyrazole, and potency regions (Fig. 1). First, we will discuss the synthetic preparation of the inhibitors. This will be followed by a disclosure of their in vitro biochemical activity and oxidative metabolic stability. Finally, in vivo reduction of cortical Aβ in *wild type* mice will be discussed.

The initial synthetic route outlined in Scheme 1 installed the aryl ring and potency piece in the first step using a reductive amination which was followed by sulfonylation. Saponification of the

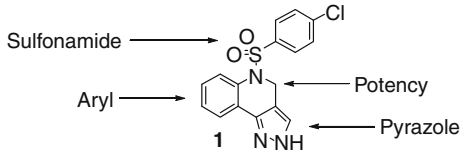
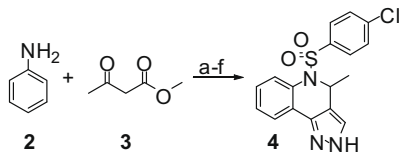


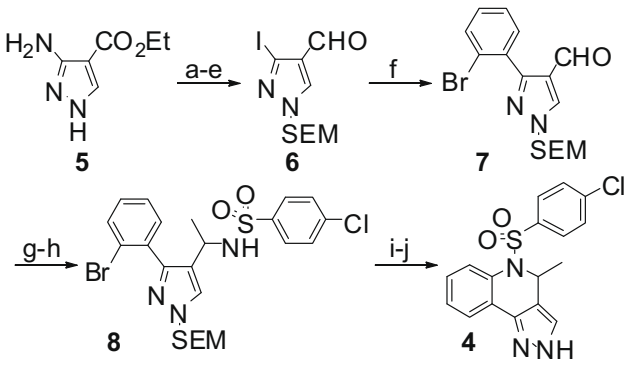
Figure 1. Representative γ-secretase inhibitor.



Scheme 1. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, 1,2-DCE; (b) 4-chlorobenzenesulfonyl chloride, pyridine; (c) aq NaOH, MeOH; (d) TFAA, TFA, reflux; (e) DMA, DMF, 110 °C; (f) hydrazine, AcOH, EtOH.

* Corresponding author.
E-mail address: gary.probst@elan.com (G.D. Probst).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.092

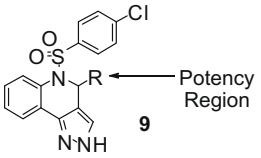


Scheme 2. Reagents and conditions: (a) isoamylnitrite CH_2I_2, 0 °C to 100 °C, 66%; (b) NaH, SEM-Cl, THF, 95%; (c) aq NaOH, MeOH; (d) BH_3-DMS, THF, 50 °C; (e) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 80% over three steps; (f) 2-bromophenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DME, H_2O, 80 °C, 75%; (g) 4-chlorobenzenesulfonamide, $Ti(O\text{-}iPr)_4$, THF; (h) MeMgBr, THF, −78 °C, 74% over two steps; (i) CuI, CsOAc, DMSO, 120 °C, 67%; (j) 4 N HCl in dioxane, MeOH, 60 °C, 100%.

ester, cyclization to the quinolone, and finally pyrazole formation completed the synthesis. Since we wished to maintain the pyrazole as a constant and vary the other three regions, this synthetic route was highly inefficient. Furthermore, this sequence was plagued by low yields and/or incompatible chemistry (e.g., the cyclization step with electron-deficient aryl rings).

A second synthetic sequence was required where the pyrazole would be introduced early with two distinct synthetic handles in order to build in a bi-directional manner which would allow for the introduction of variation into the other three regions late in the synthesis (Scheme 2). The protected pyrazole **6** bearing differentiated synthetic handles, an iodide and aldehyde, was prepared in five steps.[8] A Suzuki–Miyaura coupling with **6** and the appropriate 2-bromoarylboronic acid effectively installed the aryl portion of the quinoline.[9] Condensation of the aldehyde with the requisite

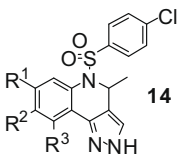
Table 1
SAR of potency region substituents[a]



#	R	IC_{50}[b] (nM)	OxMet., (m, h)[c]
1	H	27	2, 0
4	Me	1.2	16, 3
10	Et	0.36	7, 2
11	*i*Pr	0.28	2, 1
12	cPr	0.17	15, 4
13	CF_3	0.21	10, 13

[a] Compounds are racemic.
[b] Values are means of three experiments. See Ref. 11.
[c] See Ref. 12.

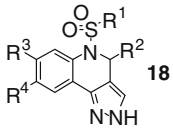
Table 2
SAR of fluoride on the aryl region[a]

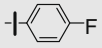
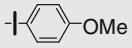
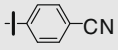
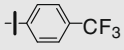
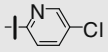
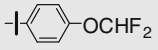
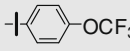
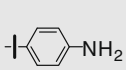
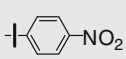


#	R^1	R^2	R^3	IC_{50}[b] (nM)	OxMet., (m, h)[c]
15	F	H	H	2.1	23, 6
16	H	F	H	1.6	23, 13
17	H	H	F	1.4	11, 3

[a] Compounds are racemic.
[b] Values are means of three experiments. See Ref. 11.
[c] See Ref. 12.

Table 3
Sulfonamide SAR[a]



#	R^1	R^2	R^3	R^4	IC_{50}[b] (nM)	OxMet., (m, h)[c]
19[d]		cPr	H	H	4.2	na
20		cPr	H	H	0.42	11, 8
21		cPr	H	H	3.2	2, 3
22		cPr	H	H	0.74	11, 9
23		cPr	H	H	0.31	49, 45
24		Me	H	H	140	3, 5
25		Me	H	H	290	46, 73
26		cPr	H	F	1.3	49, 62
27		cPr	H	F	0.76	56, 68
28		cPr	H	F	0.37	55, 60
29		Me	H	F	280	45, 76
30		Me	H	F	5500	65, 76
31		cPr	F	F	2.9	5, 1
32		cPr	F	F	0.52	9, 4
33[d]		cPr	F	F	0.46	4, 23
34		cPr	F	F	85	35, 53
35		cPr	F	F	40	na

[a] Compounds are racemic unless stated otherwise.
[b] Values are means of three experiments. See Ref. 11.
[c] See Ref. 12.
[d] Pure enantiomer.

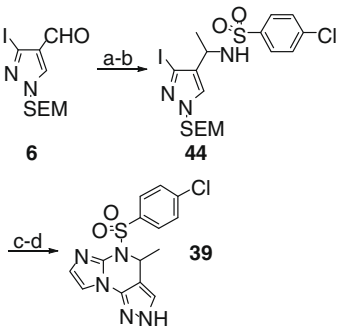
sulfonamide, and subsequent addition of the desired Grignard reagent to the sulfonylimine afforded the cyclization precursor **8**. A copper-mediated cyclization[10] followed by removal of the protecting group produced the desired compounds.

With synthetic chemistry in-hand, we endeavored to focus intensely on increasing the oxidative metabolic stability of **1** along with the potency in hopes of creating efficacious inhibitors. Our initial efforts were to explore the potency region. Placement of alkyl groups in this zone dramatically increased the potency as shown in Table 1. The larger alkyl groups generated increased potency with cyclopropyl **12** having the greatest effect with an increase in potency of over 150-fold. A trifluoromethyl group **13** afforded a similar potency with a slight increase in metabolic stability across both species.

The effect of fluorine on the aryl portion of the quinoline was explored (Table 2). Little effect was observed except for a modest increase in oxidative metabolic stability in both species with a fluorine at the 8-position of **16**.

Exploration of the sulfonamide region revealed wide fluctuations in potency and metabolic stability as shown in Table 3. The placement of lipophilic groups at the *para*-position provided approximately a 10-fold increase in potency (e.g., **12** vs **19**). Polar groups at that position had a detrimental effect on potency (**21**, **34**, and **35**), except for the nitrile **22**. Potent and metabolically stable compounds were realized with lipophilic electron-withdrawing groups[13a] at the *para*-position of the sulfonamide in conjunction with a cyclopropyl moiety[13b] as the potency piece (**23**, **27**, and **28**). Alternatively, metabolic stability could be achieved by lowering the *c* log *P* via the incorporation of nitrogen into the aryl ring of the sulfonamide **26**. Interestingly, the thiophene **32** and thiazole **33** were potent, but each proved to be metabolically unstable.

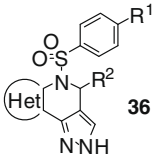
Since lowering the *c* log *P* proved to be a fruitful strategy to improve metabolic stability, further investigations were warranted on the aryl region. Unfortunately, all of the heteroaryl groups, **38–41** and **43**, resulted in a significant loss in potency except for the thiophene **37** and pyridyl **42**, but both suffered from metabolic inadequacies (Table 4). Of synthetic interest from this series is the preparation of the bridgehead nitrogen compound **39** which was prepared via a tandem N-arylation with **44** and 2-bromoimidazole (Scheme 3).

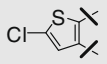
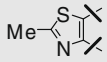


Scheme 3. Reagents and conditions: (a) 4-chlorobenzenesulfonamide, Ti(O-*i*Pr)$_4$, THF; (b) MeMgBr, THF, −78 °C, 54% over two steps; (c) 2-bromoimidazole, CuI, *trans*-*N*,*N*′-dimethylcyclohexane-1,2-diamine, Cs_2CO_3, dioxane, reflux, 70%; (d) 4 N HCl in dioxane, MeOH, 60 °C, 100%.

Finally, the structure–activity relationship on the aryl region revealed that the 7-position was highly tolerant of an eclectic mix of functionalities as shown in Table 5. This allowed for the alteration of the physiochemical properties, thus the modulation of the phar-

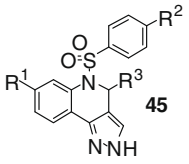
Table 4
SAR of heteroaryls[a]



#	Het	R^1	R^2	IC_{50}[b] (nM)	OxMet., (m, h)[c]
37		CF_3	Me	4.5	18, 3
38		CF_3	cPr	24	8, 31
39		Cl	Me	27	56, 74
40		CF_3	cPr	1.1	53, 59
41		CF_3	cPr	2.1	25, 44
42		Cl	Me	2.6	3, 6
43		CF_3	cPr	2.4	88, 87

[a] Compounds are racemic.
[b] Values are means of three experiments. See Ref. 11.
[c] See Ref. 12.

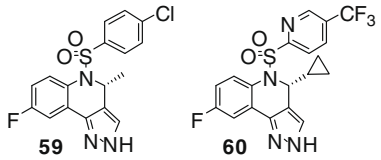
Table 5
SAR at the 7-region of the aryl region[a]



#	R^1	R^2	R^3	IC_{50}[b] (nM)	OxMet., (m, h)[c]
46	OMe	Cl	Me	2.0	1, 1
47	OBn	Cl	Me	1.5	1, 0
48	OH	Cl	Me	0.65	27, 50
49[d]	I	CF_3	cPr	0.26	78, 80
50	Cl	CF_3	cPr	0.44	85, 87
51	CN	CF_3	cPr	0.17	77, 80
52	CO_2Me	CF_3	cPr	0.26	na
53		Cl	Me	0.93	0, 0
54		CF_3	cPr	0.26	13, 32
55		CF_3	cPr	0.24	55, 59
56		CF_3	cPr	0.41	32, 60
57		CF_3	cPr	0.58	17, 29
58		CF_3	cPr	0.20	3, 27

[a] Compounds are racemic unless stated otherwise.
[b] Values are means of three experiments. See Ref. 11.
[c] See Ref. 12.
[d] Pure enantiomer.

Table 6
In vitro properties of the γ-secretase inhibitors



#	IC_{50}[a] (nM)	OxMet., (m, h)[b]	GlucMet., (m, h)[c]	P_{app}[d] (nm/s)	P-gp Efflux[e]	Sol.[f] (μM)
59	0.62	7, 1	91, 77	198	0.81	>100
60	0.67	83, 95	89, 97	249	1.47	95

[a] Values are means of three experiments. See Ref. 11.
[b] See Ref. 12.
[c] See Ref. 14.
[d] See Ref. 15.
[e] See Ref. 16.
[f] See Ref. 17.

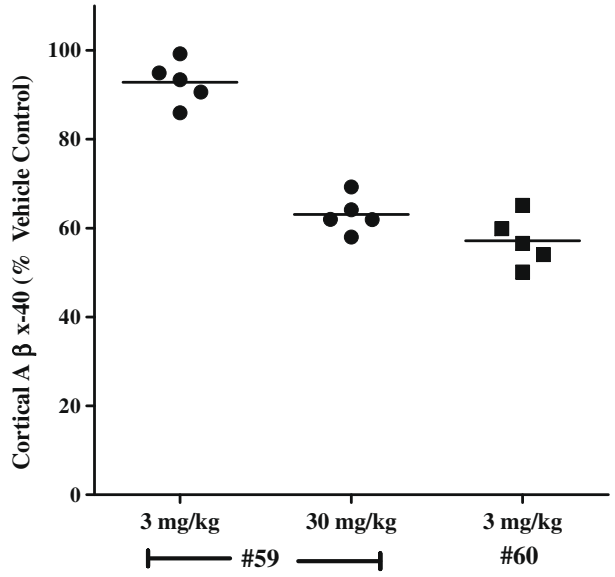


Figure 2. Reduction of cortical Aβx-40[18] after 3 h in FVB mice after a single oral dose as compared to the vehicle-dosed animals. Each circle or square represents one animal.

macokinetic parameters of these compounds. Compounds **49**–**51** displayed excellent potency and metabolic stability.

To validate the strategy of focusing on alleviating the metabolic liability, it was necessary to test two compounds in vivo with similar in vitro characteristics. Enantiomerically pure samples of compounds **59** and **60** were obtained by chiral HPLC. Their in vitro properties are nearly identical except for their oxidative stability which are 7% and 83% for **59** and **60**, respectively, in mouse liver microsomes (Table 6). Both of these compounds were tested in vivo by administration of a single PO dose to FVB mice. Compound **59** reduced Aβx-40 by 7% and 37% at 3 and 30 mpk, respectively, in the cortex, whereas compound **60** proved to be significantly more efficacious by reducing Aβx-40 by 43% at 3 mpk (Fig. 2). Our strategy of improving the oxidative metabolic stability was a major determinant that led to more efficacious compounds, although there certainly could be additional factors contributing to the increased efficacy.

In conclusion, we have discovered potent and metabolically stable γ-secretase inhibitors with potential utility as a therapy for Alzheimer's disease. These inhibitors dramatically reduced cortical Aβx-40 in FVB mice at pharmaceutically relevant oral doses.[19]

Acknowledgments

We would like to thank Jennifer Marugg, Wes Zmolek, Jackie Kwong, David Nakamura, Rose Lawler, Juri Fukada, Lany Ruslim, Michael Lee, Kang Hu, Kevin Tanaka, Terence Hui, Tracy Cole, Cristian Cabrera, Pamela Santiago, Ferdie Soriano, Brain Peterson, Ron Morgan, Jenifer Smith, John Maynard, David Quincy, and Lee Latimer for their contributions to this work.

References and notes

1. Ferri, C.; Prince, M.; Brayne, C.; Brodaty, H.; Fratiglioni, L.; Ganguli, M.; Hall, K.; Hasegawa, K.; Hendrie, H.; Huang, Y. *The Lancet* **2005**, *366*, 2112.
2. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
3. Sinha, S.; Liberburg, I. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11049.
4. (a) Sankaranarayanan, S.; Holahan, M. A.; Colussi, D., et al *J. Pharmacol. Exp. Ther.* **2009**, *328*, 131; (b) Hussain, I.; Hawkins, J.; Harrison, D., et al *J. Neurochem.* **2007**, *100*, 802; (c) Nishitomi, K.; Sakaguchi, G.; Horikoshi, Y., et al *J. Neurochem.* **2006**, *96*, 1; (d) Dovey, H. F.; John, V.; Anderson, J. P., et al *J. Neurochem.* **2001**, *76*, 173.
5. Schmidt, B.; Baumann, S.; Braun, H. A.; Larbig, G. *Curr. Top. Med. Chem.* **2006**, *6*, 377.
6. (a) Guo, T.; Gu, H.; Hobbs, D. W.; Rokosz, L. L.; Stauffer, T. M.; Jacob, B.; Clader, J. W. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3010; (b) Asberom, T.; Bara, T. A.; Clader, J. W.; Greenlee, W. J.; Guzik, H. S.; Josien, H. B.; Wei, L.; Parker, E. M.; Pissarnitski, D. A.; Song, L.; Zhang, L.; Zhao, Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 205; (c) Asberom, T.; Guzik, H. S.; Josien, H. B.; Pissarnitski, D. A., 2003. WO2003014075.
7. Neitzel, M. L.; Aubele, D. L.; Hom, R.; Konradi, A. W.; Probst, G.; Semko, C. M.; Truong, A. P.; Garofalo, A. W., 2007. WO2007143523.
8. (a) Gavina, B. D.; Forte, B.; Mantegani, S.; Varasi, M.; Vianello, P., 2005. WO2005005414.; (b) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
9. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
10. Yamada, K.; Kubo, T.; Tokuyama, H.; Fukuyama, T. *Synlett* **2002**, 231.
11. Compound, initially diluted with DMSO, was incubated with gamma secretase prepared from IMR-32 cell membranes. The reaction, at 37 °C, was initiated by the addition of MBPC-125 Swedish substrate for 2 h, and then quenched by the addition of SDS. Quantification of cleaved substrate was determined by an Aβ40 specific ELISA assay.
12. Percentage of compound (1 μM) remaining after 30 min incubation in liver microsomes (0.5 mg/ml protein) supplemented with 1 mM NADPH at 37 °C in phosphate buffer. (m = mouse, h = human).
13. (a) Smart, B. E. *J. Fluorine Chem.* **2001**, *109*, 3; (b) Manoury, P. M.; Binet, J. L.; Rousseau, J.; Lefevre-Borg, F. M.; Cavero, I. G. *J. Med. Chem.* **1987**, *30*, 1003.
14. Percentage of compound (2 μM) remaining after 30 min incubation in liver microsomes (0.5 mg protein) supplemented with 1 mM UDPGA, 100 mM $MgCl_2$ and 25 μg/mL alamethacin at 37 °C in phosphate buffer. (m = mouse, h = human).
15. Compound (5 μM) in mHBSS (pH 7.4) were incubated with MDCK II cell monolayers for 120 min at 37 °C. Samples were taken from apical and basolateral chambers, and analyzed using LC/MS/MS.
16. Compound (5 μM) in mHBSS (pH 7.4) were incubated with MDR1-MDCK cell monolayers for 120 min at 37 °C with and without a P-gp inhibitor. Samples were taken from apical (A) and basolateral (B) chambers, and analyzed using LC/MS/MS. The efflux ratio was determined by dividing the rate of the A to B direction with and without a P-gp inhibitor.
17. Compound (20 mM) were serial diluted in DMSO and aliquoted to buffer (HBSS pH 7.4), 1:200 dilution. After 1 h incubation, plates were read on a solubility scanner.
18. Zhou, B.; Yu, M.; Neitzel, M.; Marugg, J.; Jagodzinska, J.; Lee, M.; Hu, K.; Schenk, D.; Yednock, T.; Basi, G. *J. Biol. Chem.* **2008**, *283*, 2927.
19. Details of in vivo and in vitro experiments will be published separately.