Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 511–516
Bioorganic & Medicinal Chemistry Letters

Discovery of γ-secretase inhibitors efficacious in a transgenic animal model of Alzheimer's disease

Theodros Asberom,[a] Zhiqiang Zhao,[a] Thomas A. Bara,[a] John W. Clader,[a] William J. Greenlee,[a] Lynn A. Hyde,[b] Hubert B. Josien,[a] Wei Li,[a] Andrew T. McPhail,[c] Amin A. Nomeir,[d] Eric M. Parker,[b] Murali Rajagopalan,[a] Lixin Song,[b] Gwendolyn T. Wong,[b] Lili Zhang,[b] Qi Zhang[b] and Dmitri A. Pissarnitski[a,*]

[a]*Department of Chemical Research, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*
[b]*Department of Central Nervous System, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*
[c]*Department of Chemistry, Duke University, Durham, NC 27708-0346, USA*
[d]*Department of Drug Metabolism and Pharmacokinetics, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*

Received 31 August 2006; revised 5 October 2006; accepted 5 October 2006
Available online 10 October 2006

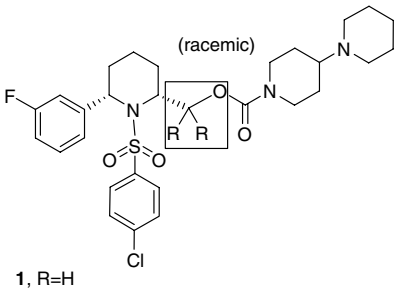
Abstract—Attachment of the cyclopropylcarbamate group to the piperidine core of γ-secretase inhibitors leads to a dramatic increase of their in vitro potency. Strategies for subsequent improvement of the in vivo pharmacokinetic profile of the series are discussed. Resulting compounds significantly reduce Aβ levels in TgCRND8 mice after a single PO dosing at 30 mpk.

The most common degenerative brain disorder, Alzheimer's disease (AD), makes up to 70% of all cases of dementia and is the third most common cause of death in the United States.[1] One of the major pathological hallmarks of AD is abnormal extracellular deposition of β-amyloid peptide (Aβ) in the form of plaques in the brain of AD patients. Although the exact cause of AD is unknown, a large body of evidence suggests[2] that overproduction of amyloid peptides, especially $A\beta_{42}$, is central to its pathogenesis. Amyloid peptides are produced by proteolysis of the amyloid precursor protein (APP) by sequential action of β- and γ-secretases. Because of its central role in the generation of Aβ peptide, γ-secretase was proposed as a target for the treatment of AD.[3]

Previously we described a series of γ-secretase inhibitors related to the sulfonylated piperidine **1**.[4] Compound **1** was assessed in vivo in a transgenic animal model of AD based on TgCRND8 mice,[5] but its ability to reduce $A\beta_{40}$ in the plasma was significant only when dosed subcutaneously at 100 mpk. We hypothesized that the low efficacy of **1** was due to poor PK, possibly resulting from the rapid in vivo hydrolysis of the carbamate linker. In order to slow down this metabolic process via the introduction of the steric hindrance, molecule **2** featuring a *gem*-dimethyl group was designed. We were pleased to see that not only was the AUC[6] of **2** increased by nearly a factor of 2, but also the potency of **2** was also enhanced by several fold. Despite the improvement in the metabolic stability of **2**, its chemical stability in the acidic media was not adequate. The compound decomposed on the attempt to formulate it as a hydrochloric salt using 1 M HCl. This outcome is unsurprising in the view of the similarity between the carbamate fragment of **2** and BOC protecting group, known to be cleavable under acidic pH. We reasoned that by formation of an additional bond between the two carbon atoms of the *gem*-dimethyl group, thus converting it to the cyclopropyl moiety, stability of the compound to the acid would be increased.

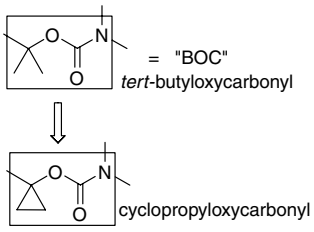
Keywords: Alzheimer's disease; γ-Secretase inhibitors; Kulinkovich reaction.
* Corresponding author. E-mail: Dmitri.Pissarnitski@spcorp.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.10.011



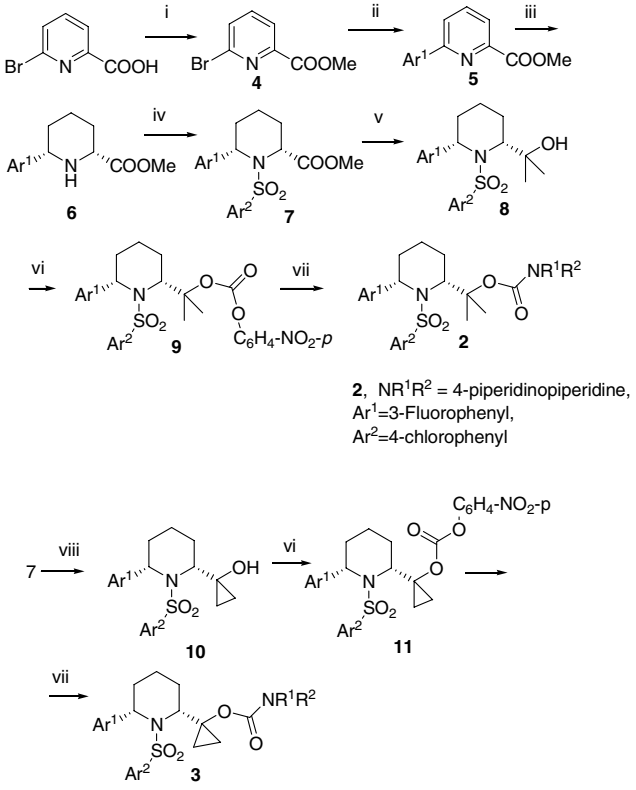
1, R=H
Membr. Ab40 IC50 = 11.2 nM
Rat AUC $_{0\text{-}6\ h}$ = 1376 hr.ng/mL (@10 mpk)

2, R=Me
Membr. Ab40 IC50 = 4.4 nM
Rat AUC $_{0\text{-}6\ h}$ = 2429 hr.ng/mL (@10 mpk)



Synthesis of **2** and its cyclopropyl analogs **3** is depicted on Scheme 1. Commercial 2-bromopicolinic acid was esterified using methanol and thionyl chloride to provide ester **4**. Suzuki coupling of **4** with arylboronic acids $Ar^1B(OH)_2$ was conducted using sodium carbonate as the base in the 6:3 mixture of toluene and ethanol as the solvent at 90 °C. Resulting methyl ester **5** had up to 10% contamination of ethyl ester stemming from the transesterification with the solvent. However, without the ethanol or when it was replaced with methanol, the reaction completely failed. Catalytic hydrogenation of the pyridine ring took precedence over the hydrogenation of Ar^1 providing piperidine **6** selectively. Sulfonylation of the latter leading to **7** was achieved with arenesulfonylchlorides at elevated temperatures in pyridine as the solvent. Reaction of **7** with excess of methyl Grignard reagent in THF furnished tertiary alcohol **8**, which was converted to 4-nitrophenyl carbonate **9**. Final compound **2** was obtained from **9** by the displacement of 4-nitrophenol by 4-piperidinopiperidine. Cyclopropylcarbonates of type **3** were obtained in a similar manner from alcohol **10**, available in a good yield (72%) from ester **7** by Kulinkovich cyclopropanation.[7] Slow addition of methylmagnesium bromide to a chilled mixture of the ester **7** and titanium tetraisopropoxide in THF was essential for the success of this transformation.

Due to the steric hindrance, transformation of **11** to **3** at room temperature was impractically slow, especially with crowded amines HNR^1R^2. We screened a number of solvents to amend the process for parallel synthesis and found that the majority of reactions were completed in THF after an overnight stirring at 80 °C. Although the use of DMF as the solvent resulted in shorter reaction times, the products were often contaminated by dimethylaminocarbonate **3** ($NR^1R^2 = NMe_2$), where the dimethylamino group likely originated from a molecule of DMF.



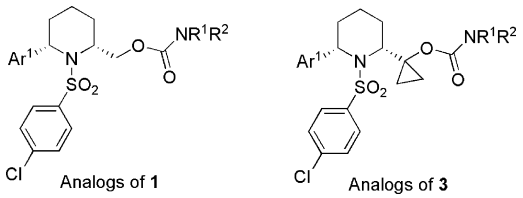
Scheme 1. Reagents and conditions: (i) MeOH, $SOCl_2$, 0 °C to reflux, 5 h; (ii) $Ar^1B(OH)_2$, cat $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH = 6:3, 90 °C, 10 h; (iii) H_2, cat PtO_2, MeOH/AcOH = 4:1; (iv) Ar^2SO_2Cl, Pyr, 70 °C, overnight; (v) MeMgBr, THF; (vi) 4-nitrophenylchlorocarbonate (2 equiv), pyridine (3 equiv), CH_3CN/THF = 1:3, 1–3 days; (vii) HNR^1R^2, THF, 80 °C, 12 h; (viii) EtMgBr (3 equiv), $Ti(OPr\text{-}i)_4$ (0.3 equiv), THF, 0 °C.

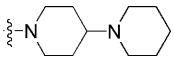
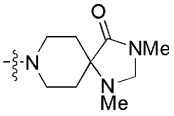
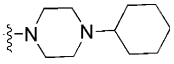
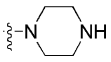
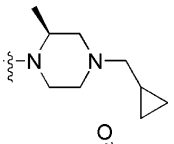
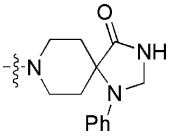
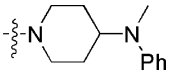
Initial profiling of the biological activity of cyclopropyl carbonates **3** using membrane-based preparations γ-secretase[8] for IC_{50} determinations demonstrated an improvement of potency by a factor of 5–18 as compared to the previously described[4] analogs of **1** (Table 1).

As was described previously, both NMR data and ab initio calculations point to the chair-like conformation of the piperidine ring in **1** with both side chains occupying diaxial positions.[4] In the course of the present work, we were able to obtain X-ray quality crystals of compound **11** (Ar^1 = 3-chlorophenyl, Ar^2 = 4-chlorophenyl). As indicated by X-ray analysis, the presence of the cyclopropyl moiety did not drastically alter the overall shape of the inhibitors (Fig. 1) and the bis-diaxial chair-like conformation was preserved.[9]

While Table 1 lists the data for the inhibition of $A\beta_{40}$ production measured using membrane-based preparations of γ-secretase,[8] it was interesting to assess the cell permeation properties of the series in the assays utilizing whole cells. We were pleased to see that most of the compounds preserved the potency well upon the transfer

Table 1. Influence of the cyclopropyl group on the potency of inhibitors[a]



Ar^1	NR^1R^2	Analogs of **1** $A\beta_{40}$ IC_{50} (nM)	Analogs of **3** $A\beta_{40}$ IC_{50} (nM)	Potency ratio
3-F–Ph		11.2	0.7	16.0
3,5-Di-F–Ph		26.8	5.3	5.0
3,5-Di-F–Ph		36.1	4.2	8.5
3,5-Di-F–Ph		74.8	7.9	9.4
3,5-Di-F–Ph		110.0	6.1	17.9
3,5-Di-F–Ph		152.5	8.5	18.0
3-F–Ph		164.5	15.4	10.7

[a] All tested compounds were racemic mixtures.

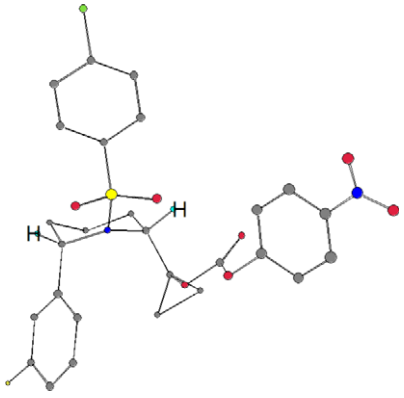
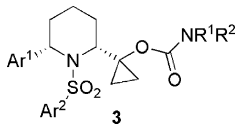


Figure 1. X-Ray crystal structure of the compound **11** (Ar^1 = 3-fluorophenyl, Ar^2 = 4-chlorophenyl). Equatorial hydrogen atoms are shown for clarity.

from the membrane to the whole cell-based assay.[10] However, molecules containing piperazines with a free NH group on the right-hand side (**19**, **24**, and **25**) showed a larger relative shift in potency as compared with N-alkylated piperazines (**20** and **27**). For the majority of the compounds, inhibition of $A\beta_{42}$ production followed the trend of $A\beta_{40}$, with potency remaining in the single- or low double-digit nanomolar range.

Preliminary information on the pharmacokinetics of the series in the rat was obtained by analysis of the 6 h AUC after oral dosing at 10 mpk (Table 2).[6] When the NR^1R^2 group lacked a second basic nitrogen (e.g., **12** and **13**), the compounds did not have any measurable plasma levels. For other compounds, the following strategies to improve PK were successfully used: (a) blocking metabolic 'hot spots' with fluorine atoms (**15**); (b) introduction of a methyl group or a bridge to create steric hindrance in the vicinity of the carbamate linker (**16** and **21**); (c)

Table 2. In vitro data and $AUC_{0-6\,h}$ after PO dosing at 10 mpk in rat[a]



3

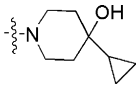
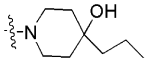
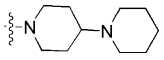
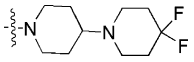
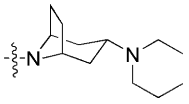
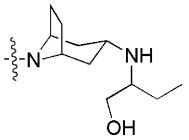
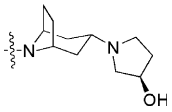
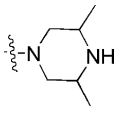
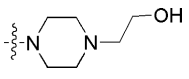
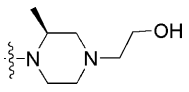
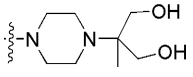
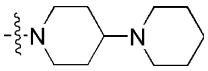
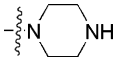
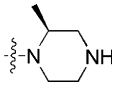
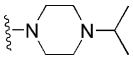
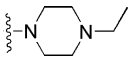
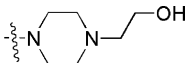
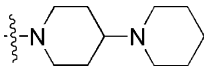
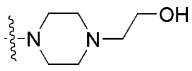
Compound	Ar^1	Ar^2	NR^1R^2	Aβ40 membrane (nM)	Aβ40 cell (nM)	AUC (h ng/mL)
12	3-F–Ph	4-Cl–Ph		3.7	7.7	0
13	3-F–Ph	4-Cl–Ph		4.5	10.7	0
14	3-F–Ph	4-Cl–Ph		0.8	5.0	802
15	3-F–Ph	4-Cl–Ph		2.3	5.5	1668
16	3-F–Ph	4-Cl–Ph		2.6	9.7	2878
17	3-F–Ph	4-Cl–Ph		5.2	21.5	3072
18	3-F–Ph	4-Cl–Ph		3.6	20.0	2090
19	3-F–Ph	4-Cl–Ph		5.3	120.4	1811
20	3-F–Ph	4-Cl–Ph		1.9	7.0	2087
21	3-F–Ph	4-Cl–Ph		2.1	28.7	7209
22	3-F–Ph	4-Cl–Ph		0.9	5.1	1768

Table 2 (*continued*)

Compound	Ar^1	Ar^2	NR^1R^2	Aβ40 membrane (nM)	Aβ40 cell (nM)	AUC (h ng/mL)
23	3,5-Di-F–Ph	4-Cl–Ph		0.3	5.8	1076
24	3,5-Di-F–Ph	4-Cl–Ph		7.9	106.0	6206
25	3,5-Di-F–Ph	4-Cl–Ph		16.8	153.5	N/A
26	3,5-Di-F–Ph	4-Cl–Ph		2.7	17.0	783
27	3,5-Di-F–Ph	4-Cl–Ph		1.1	7.5	1401
28	3,5-Di-F–Ph	4-Cl–Ph		2.4	5.0	5001
29	3,5-Di-F–Ph	3,5-Di-F–Ph		3.5	12.9	5053
30	3,5-Di-F–Ph	3,5-Di-F–Ph		16.8	24.7	23064

[a] All tested compounds were racemic mixtures.

pre-oxidation of the side chain by introduction of a hydroxyl (**20**, **21**, and **28**); (d) replacement of 4-chlorophenylsulfonyl Ar^2 group on the bottom part of the molecule with alternative patterns of fluorination (**29** and **30**). 3,5-Difluorinated phenyl as the Ar^1 substituent often provided compounds with enhanced potency and metabolic stability as compared to their monofluorinated counterparts (e.g., **28** vs **20**).

Figure 2 illustrates the efficacy of five representative members of the series in the transgenic animal model of AD. Young (6 weeks of age) TgCRND8 mice[5] which have not yet developed the amyloid plaques were used in the experiments. A group of four mice was used per each compound with a vehicle-treated group as the negative control. Following oral dosing at 30 mpk, a substantial reduction of $A\beta_{40}$ was observed in the plasma of the drug-treated mice after a 3 h period of time. The remain-

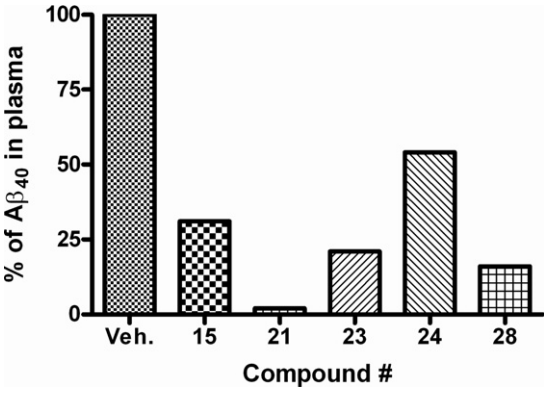


Figure 2. Reduction of plasma $A\beta_4$ in TgCRND8 mice after an oral dosing at 10 mpk as compared to the vehicle-dosed animals. Each bar represents an average of four animals.

ing levels of the $A\beta_{40}$ constituted 2–52% of those observed in the vehicle-treated group. Reduction of the levels of both $A\beta_4$ and $A\beta_{42}$ in the cortex of the brain tracked well with the effect in the plasma (data not shown and will be published separately).[11]

In conclusion, we discovered a novel series of γ-secretase inhibitors potentially useful as a therapy for the Alzheimer's disease. Several representative compounds of the series were tested in TgCRND8 mouse serving as the animal model of AD and demonstrated the ability to reduce Aβ levels in vivo.

Acknowledgments

The authors are thankful to Drs. A. Buevich, T.-M. Chan, R. Osterman, and A. Evans for NMR analyses, Dr. J. Voigt for molecular modeling and valuable discussions, and the group of Dr. J. Wong for the scale up of synthetic intermediates.

References and notes

1. Ewbank, D. C. *Am. J. Public Health* **1999**, *89*, 90.
2. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
3. Josien, H. *Curr. Opin. Drug Discov. Devel.* **2002**, *5*, 513.
4. Pissarnitski, D. A.; Asberom, T.; Bara, T. A.; Buevich, A. V.; Clader, J. W.; Greenlee, W. J.; Guzik, H. S.; Josien, H. B. W.; McEwan; McKittrick, B. A.; Nechuta, T. L.; Sinning, L.; Smith, E. M.; Parker, E. M.; Vaccaro, H. A.; Voigt, J. H.; Zhang, L.; Zhao, Z. *Bioorg. Med. Chem. Lett.* **2006**, doi:10.1016/j.bmcl.2006.09.094.
5. Chishti, M. A.; Yang, D. S.; Janus, C.; Phinney, A. L.; Horne, P.; Pearson, J.; Strome, R.; Zuker, N.; Loukides, J.; French, J.; Turner, S.; Lozza, G.; Grilli, M.; Kunicki, S.; Morissette, C.; Paquette, J.; Gervais, F.; Bergeron, C.; Fraser, P. E.; Carlson, G. A.; George-Hyslop, P. S.; Westaway, D. *J. Biol. Chem.* **2001**, *276*, 21562.
6. Following the oral dosing at 10 mpk in HPBCD or MC, AUC in rats was measured over a period of 6 h, using cassette-accelerated rapid rat screen (CARRS): Korfmacher, W. A.; Cox, K. A.; Ng, K. J.; Veals, J.; Hsieh, Y.; Wainhaus, S.; Broske, L.; Prelusky, D.; Nomeir, A.; White, R. E. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 335.
7. (a) Lysenko, I. L.; Kulinkovich, O. G. *Russ. J. Org. Chem.* **2001**, *37*, 1238; (b) Racouchot, S.; Sylvestre, I.; Ollivier, J.; Kozyrkov, Y. Y.; Pukin, A.; Kulinkovich, O.; Salauen, J. *Eur. J. Org. Chem.* **2002**, *13*, 2160.
8. Zhang, L.; Song, L.; Terracina, G.; Liu, Y.; Pramanik, B.; Parker, E. *Biochemistry* **2001**, *40*, 5049.
9. Crystallographic data have been deposited with Cambridge Crystallographic Data Centre as supplementary publication number CCDC 619153.
10. Efficacy of γ-secretase inhibitors in intact cells was measured using HEK293 cells expressing human APP with both Swedish and London mutations. The cells were grown in 96-well plate with 100 μl media per well, and were changed to fresh media and incubated with γ-secretase inhibitor for 4 h. Ten micro litres of conditioned media was used to measure $A\beta_{40}$ using ECL technology as described in Ref. 8.
11. Hyde, L. A.; Zhang, L., in preparation.