Bioorganic & Medicinal Chemistry Letters 19 (2009) 1366–1370

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Macrocyclic peptidomimetic β-secretase (BACE-1) inhibitors with activity in vivo

Rainer Machauer *, Kurt Laumen, Siem Veenstra, Jean-Michel Rondeau, Marina Tintelnot-Blomley, Claudia Betschart, Anne-Lise Jaton, Sandrine Desrayaud, Matthias Staufenbiel, Sabine Rabe, Paolo Paganetti, Ulf Neumann

Novartis Institutes for BioMedical Research, Novartis Pharma AG, PO Box, CH-4002 Basel, Switzerland

ARTICLE INFO

Article history:
Received 24 November 2008
Revised 14 January 2009
Accepted 15 January 2009
Available online 22 January 2009

Keywords:
Alzheimer's disease
BACE-1 inhibition
Macrocycles
Ethanolamine-transition state mimetic
In vivo efficacy in APP51/16 mice

ABSTRACT

The macrocyclic peptidic BACE-1 inhibitors **2a–c** show moderate enzymatic and cellular activity. By exchange of the hydroxyethylene- to ethanolamine-transition state mimetic the peptidic character was reduced, providing the highly potent and selective inhibitor **3**. Variation of the P′ moiety resulted in the macrocyclic inhibitor **14**. Both macrocycles show inhibition of BACE-1 in the brain of APP51/16 transgenic mice, **3** (**NB-544**) after intravenous and **14** (**NB-533**) after oral application.

In 1907 Alois Alzheimer described the symptoms and the pathology of a progressive, and ultimately lethal, neurodegenerative disorder, which was later named Alzheimer's disease (AD).[1] In the last three decades the pathological hallmarks of AD, amyloid plaques and neurofibrillary tangles, were extensively characterized and the major component of the plaques identified as the β-amyloid peptides ($A\beta_{40/42}$).[2] The β-amyloid hypothesis describes the pathogenic mechanisms leading to AD.[3] According to this commonly accepted model, $A\beta_{4042}$ aggregates are neurotoxic and initiate a cascade of events resulting in neurodegeneration and dementia. The β-amyloid peptides, mostly 40 or 42 residues in length, are generated from the β-amyloid precursor protein (APP) by sequential proteolytic action by the β- and γ-secretase enzymes.[4] Inhibition of the membrane-bound aspartyl protease β-secretase (BACE-1, β-site APP cleaving enzyme) is considered to be one of the most promising therapeutic approaches for AD,[5] since BACE-1 knock-out/APPtransgenic mice lack $A\beta_{40/42}$, do not form amyloid plaques and are viable.[6]

By structure-based design we have modified the peptide like lead structure **1** into a potent macrocyclic inhibitor of BACE-1 (**2c**, Fig. 1).[7]

The macrocycles **2a–c** show moderate to good inhibitory potencies of 0.59 μM (**2a**), 0.25 μM (**2b**) and 0.15 μM (**2c**) when tested in the BACE-1 enzymatic activity assay, but exhibit significantly lower potencies of >10 μM (**2a,b** 16% and 27% inhibition at 10 μM) and

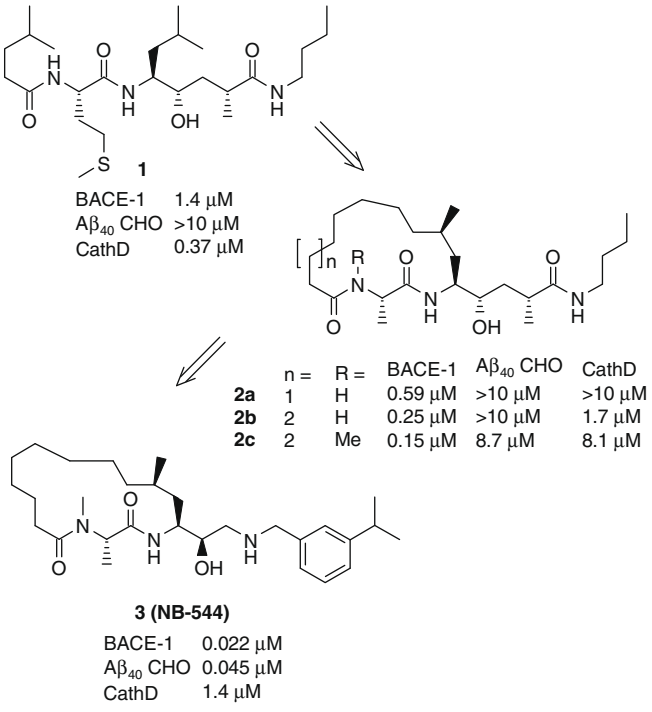


Figure 1. Development of peptidic BACE-1 inhibitor **1** into the macrocyclic inhibitor **3**.

* Corresponding author. Tel.: +41 61 6963431; fax: +41 61 6962455.
E-mail address: rainer.machauer@novartis.com (R. Machauer).

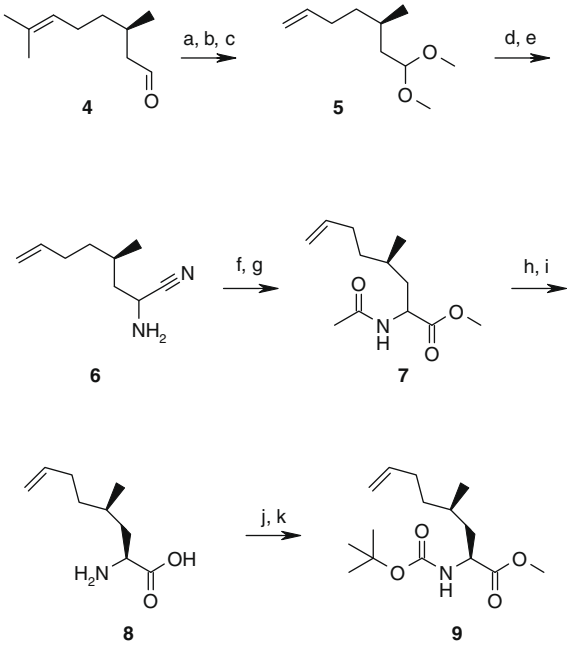
0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.01.055

8.7 μM (**2c**) in a cellular system.[7] During the course of our project other groups published on related approaches for macrocyclic BACE-1 inhibitors.[8]

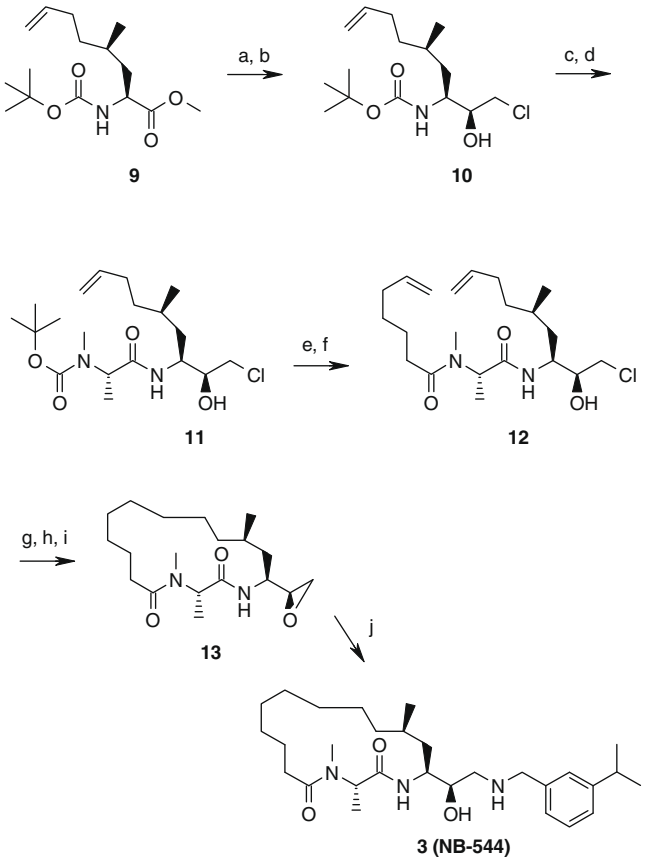
Since **2a–c** still comprise three amide bonds that confer to the BACE-1 inhibitors a peptidic character, we suspected that this was the reason for the discrepancy between the enzymatic and the cellular potency. *N*-methylation of the P2/3 amide was not sufficient to reduce the peptidic character of **2c**, nevertheless an improvement of the enzymatic activity of **2c** over **2b** was achieved.[7] Thus a further reduction of the peptidic character of the macrocyclic inhibitors may improve their cellular potency, ultimately leading to inhibitors with potential for in vivo activity.

We therefore considered options to further reduce the number of amide bonds, and the number of H-bond donors and acceptors. Based on the successful switch from the hydroxyethylene- to ethanolamine-transition state in another series of BACE-1 inhibitors (unpublished results) we designed compound **3** (Fig. 1) as our next synthetic target. A completely new synthesis was necessary for this macrocycle. The chemo-enzymatic reaction sequence to the intermediate non-natural amino acid **8** is depicted in Scheme 1, the preparation of the macrocyclic core is outlined in Scheme 2.

Commercial (*R*)-citronellal was subjected to acetalisation, ozonolysis and methyl Wittig reaction to afford **5** in good yield. Deprotection of the aldehyde and Strecker reaction provided aminonitrile **6** as diastereomeric mixture. The amine was acetylated and the nitrile converted into the methyl ester **7**. The direct hydrolysis of the aminonitrile **6** to the racemic amino acid was not successful, causing the retro-Strecker reaction. Enzymatic kinetic resolution of **7** with Alcalase (protease from *Bacillus licheniformis*),[9] at pH 7.5 under pH-stat conditions afforded the saponified (*S*)-acetyl-amino acid, whereas the unreacted (*R*)-acetyl methyl ester was easily separated by extraction from the reaction mixture. Alcalase was chosen for the resolution because of its usually high enantioselectivity against non-proteinogenic racemic amino acid derivatives and remarkably broad substrate tolerance. The efficiency of this method has been demonstrated previously in the enantioselective hydrolysis of meta-tyrosine, mono-, di- or trisubstituted phenylanilines and even *tert*-leucine.[10] In fact, this protease only recognized the chirality / stereochemistry at the α-position of the methyl ester **7**. Subsequent acetyl hydrolysis of the remaining aqueous phase catalyzed by aminoacylase from *Aspergillus* sp. (Amano acylase) provided amino acid **8**.[11] The acetyl cleavage was done enzymatically since first attempts to deprotect the amine by treatment with hydrochloric acid led to partial addition of hydrochloric acid to the terminal double bond. The (*R*)-acetyl methyl ester was recycled to **7** by equilibration with sodium *tert*-butoxide. Protection of the amino function of **8** employing di-*tert*-butyl dicarbonate (Boc_2O) and methyl ester formation afforded the non-natural amino acid **9**.



Scheme 1. Reagents and conditions: (a) $HC(OMe)_3$, MeOH, cat. H^+; (b) O_3, $NaHCO_3$, PPh_3; (c) $Ph_3P=CH_2$, 83% for three steps; (d) TFA/H_2O; (e) NH_4Cl, NaCN, NH_3, MeOH, 70% for two steps; (f) AcCl, i-Pr_2NEt, CH_2Cl_2; (g) HCl, MeOH, H_20, 37% for two steps; (h) Alcalase®; (i) Acylase®, 50% for two steps; (j) Boc_2O, Na_2CO_3, H_2O, 95%; (k) MeI, $KHCO_3$, DMF, 88%.



Scheme 2. Reagents and conditions: (a) ICH_2Cl, LDA, THF, −78 °C; (b) $NaBH_4$, EtOH, −78 °C, 83% for two steps; (c) HCl in Et_2O, CH_2Cl_2, (d) Boc-NMe-Ala-OH, i-Pr_2NEt, HOBt, EDC, CH_2Cl_2, 91% for two steps; (e) HCl in Et_2O, CH_2Cl_2, 95%; (f) 6-heptenoic acid, i-Pr_2NEt, HOBt, EDC, CH_2Cl_2, 74%; (g) Grubbs2, CH_2Cl_2, 50 °C, 83%; (h) H_2, Pd-C, EtOH, 83%; (i) NaOH, THF, MeOH, 87%; (j) 3-isopropyl benzylamine, 50 °C, 38–79%.

The protected amino acid **9** was converted into chlorohydrin **10** (diastereoselectivity approx. 5:1). Removal of the Boc protecting group and peptide coupling with Boc-*N*-methyl alanine afforded **11** in excellent yield. Deprotection of the amino-terminus and coupling with 6-heptenoic acid provided the metathesis precursor **12**. The ring closure was accomplished employing Grubbs second generation catalyst.[12] The obtained E/Z mixture of the macrocyclic olefin was hydrogenated in the presence of palladium on charcoal, followed by epoxide formation to afford the envisioned macrocyclic core **13**. Aminolysis of the epoxide with a small set of substituted benzylamines (data not shown) provided macrocycle **3** as the most potent example.

Macrocycle **3** was a potent BACE-1 inhibitor with an IC_{50} of 27 nM in the enzymatic assay, 5-fold more active than **2c**. In the human APP-transfected CHO cell assay, **3** inhibited the release of

$A\beta_{40}$ with an IC_{50} of 45 nM. This represents a 190-fold improvement over **2c**. Furthermore, **3** displayed an IC_{50} on human cathepsin D (CathD) of only 1.4 μM, corresponding to a 53-fold selectivity over this aspartyl protease (Table 1).[13].

A co-crystal of BACE-1 with **3** was obtained,[15] the structure was determined at 2.1 Å resolution and compared with the X-ray structure of **2a**.[7] Compound **3** and **2a** differed in the size of their macrocycle (16- vs 15-membered ring), in the *N*-methylation of the P2–P3 peptide bond, and they featured distinct transition state mimics and P′ groups. In spite of these significant differences, the overall BACE-1 binding mode of **3** was similar to **2a**, although 0.5–1.0 Å shifts in atomic positions were observed for equivalent inhibitor atoms and some interacting active site residues (Fig. 2). The larger macrocycle of **3** made tighter hydrophobic contacts to Ile118, Leu30 and Trp115 than its counterpart in **2a.** The *N*-methylation had virtually no influence on the orientation of the peptide bond, the H-bonded interaction between the amide carbonyl oxygen and Thr232 N was maintained, but a short contact between the methyl group and Gln73 O led to a slightly more open conformation of the enzyme flap. Nevertheless, the hydrogen-bonded contacts between the P1–P2 amide, Gly230 and the flap were very similar in both complexes. The ethanolamine-transition state mimic was found to engage in the expected short contacts between the secondary alcohol and Asp32 Oδ2 (2.6 Å) and between the amine nitrogen, Asp228 Oδ2 (2.6 Å) and Gly34 O (3.2 Å). Like the *n*-butylamino group of **2a**, the lipophilic isopropylbenzyl moiety of **3** was bound into the P2′ subsite, lined on one side by the flap residues Val69, Pro70, Tyr71 and Thr72 and, on the other side, by Tyr198. In addition to hydrophobic contacts to Ser35, Val69, Tyr71 and Tyr198, the main merit of this P′ group probably resided in its rigidity, since the position and orientation of the benzyl ring was not optimal for strong π–stacking interactions with either Tyr71 or Tyr198.

Because of its good enzymatic and cellular potency combined with good selectivity over CathD, BACE-1 inhibitor **3** was an excellent candidate for in vivo testing. Compound **3** was therefore dosed in C57BL/6 mice and compound levels in brain and plasma were determined. With an i.v. dose of 30 μmol/kg (~15 mg/kg), the brain concentration was maintained above 1 μM over a period of 5 h. The brain to plasma ratio was 1.5:1 at 5 min and reached a ratio of 4:1 at 5 h post-administration (Fig. 3).

The in vivo efficacy of compound **3** was investigated in APP51/16 transgenic mice expressing wild-type human APP under a brain specific promotor.[16] Inhibitor **3** was applied i.v. at 30 μmol/kg and the animals were sacrificed 5 h after the dosing. Another group of mice received two applications of compound **3** with a 2 h interval between the two doses and was sacrificed 3 h after the last dose. Inhibitor **3** was detected in the mouse brains of the single dose experiment and at 1.3 μmol/kg and at 4.3 μmol/kg of the double dose experiment. Forebrain extracts were analyzed and the direct BACE-1-dependent APP metabolite C_{99} as well as the α-secretase product C_{83} were quantified by Western blot while $A\beta_{40}$ and $A\beta_{42}$ were immunoprecipitated and quantified by MALDI-TOF as described.[16] While no significant effects were observed in the group treated with a single dose of compound **3** (data not shown), a significant 31% reduction of C_{99} was observed after the double administration. Furthermore, both $A\beta_{40}$ (−14%) and $A\beta_{42}$ (−20%, $p = 0.006$) were significantly reduced (Table 2 and Fig. 5).

According to the APP processing Scheme (Fig. 4) we expected that the reduction of the APP processing via the amyloidogenic pathway (BACE-1) might be compensated by a corresponding increase of the non-amyloidogenic pathway (via α-secretase). Indeed, analysis of the α-secretase product C_{83} showed a

Table 1
Enzymatic and cellular inhibition assay results and in vitro permeation data of the macrocycles **2a–c**, **3** and **14**

Compound	hBACE-1 FQ IC_{50}, μM[a]	$A\beta_{40}$ CHO IC_{50}, μM[a]	hCathD FQ IC_{50}, μM[a]	MDCK mdr1a BA/AB
2a	0.59	>10	>10	n.a.
2b	0.25	>10	1.7	n.a.
2c	0.15	8.7	8.1	n.a.
3	0.027	0.045	1.4	15
14	0.002	0.024	0.001	9.4

IC_{50} values for BACE-1 and cathepsin D inhibition, and inhibition of cellular release of $A\beta_{40}$ were determined as already described.[7] The permeation across membranes and efflux were determined in a system described in the literature.[14]

[a] Values are means of at least three experiments, n.a. = not available.

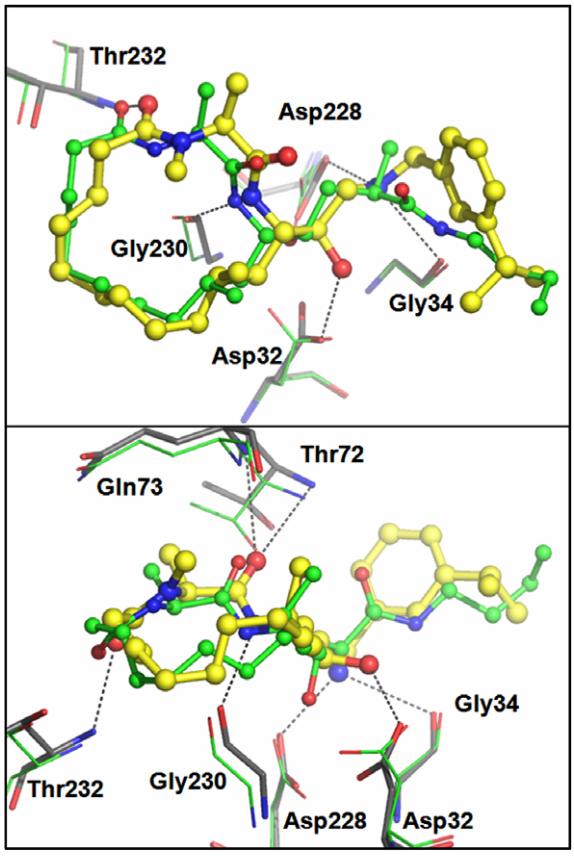


Figure 2. Two orthogonal views of the overlay of the BACE-1 complexes with **2a** and **3**.

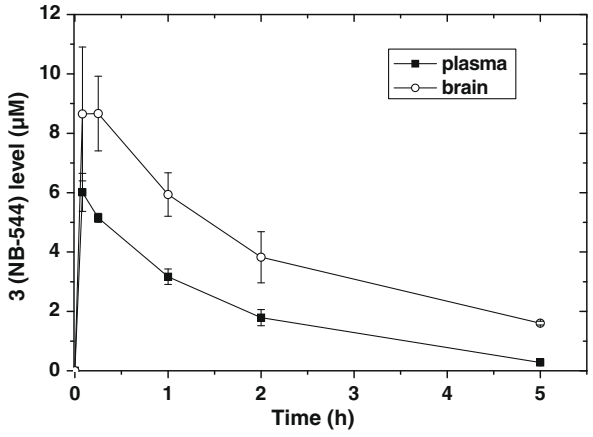


Figure 3. Plasma and brain concentrations of **3** in C57BL/6 mice over the course of 5 h (dosing: 30 μmol/kg, i.v.).

Table 2
Reduction of $A\beta_{40}$ and C_{99} in vivo by inhibitor **3**

Dose	Route	PSC833	Reduction in %		
[μmol/kg]		[μmol/kg]	$A\beta_{40}$ Brain	$A\beta_{40}$ CSF	C_{99} Brain
2 × 30	i.v.	–	−14 ($p < 0.003$)	n.a.	−31 ($p < 0.001$)
30	i.v.	25	−29 ($p < 0.001$)	−55 ($p < 0.001$)	−58 ($p < 0.003$)
300	p.o.	25	−31 ($p < 0.001$)	n.a.	−46 ($p < 0.001$)

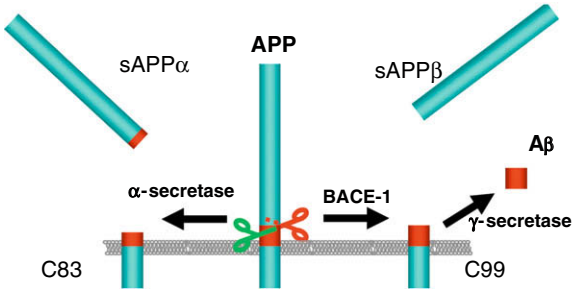


Figure 4. Processing of the β-amyloid precursor protein (APP) by α-secretase, BACE-1 and γ-secretase.

significant increase in the amount of this APP metabolite in the brain (28%, $p = 0.007$) (Fig. 5).

These data taken together demonstrate that inhibitor **3** reduced the amyloidogenic processing of APP in favor of increased proteolytic cleavage of APP by the non-amyloidogenic pathway. This evidence qualified compound **3** as a genuine BACE-1 inhibitor in vivo.

During the profiling of **3** we recognized the P-glycoprotein (P-gp) susceptibility of the compound (BA/AB = 15) in MDCK cells stably transfected with the human mdr1a gene.[14] Therefore, we conducted an efficacy study in the presence of the potent P-glycoprotein inhibitor PSC833 (25 μmol/kg, p.o., 1 h before application of the BACE-1 inhibitor).[17] Macrocycle **3** was dosed once i.v. at 30 μmol/kg and the animals were sacrificed 4 h later. The brain levels of inhibitor **3** (4.7 μmol/kg) were significantly higher than in the absence of PSC833 and in the same range as observed in the double dosing experiment. Analysis of the brain extracts showed a much stronger reduction of $A\beta_{40}$ (−29%) and C_{99} (−58%). We also analyzed $A\beta_{40}$ in the CSF of these mice, and found a strong reduction (−55%).

Given these positive results we decided to test the efficacy of inhibitor **3** also by oral administration at a single dose of

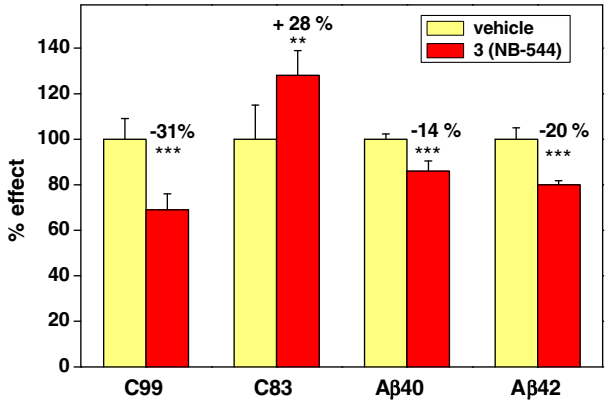
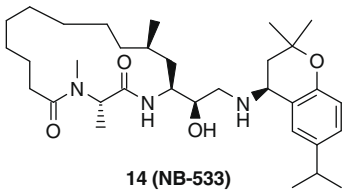


Figure 5. Analysis of the APP processing products C_{99}, C_{83}, $A\beta_{40}$ and $A\beta_{42}$ in APP51 mice after double i.v. application of inhibitor **3** (yellow bars: vehicle control, red bars: 2 × 30 μmol/kg).



BACE-1 0.002 μM
$A\beta_{40}$ CHO 0.024 μM
CathD 0.001 μM
efflux ratio 9.4

efficacy in tg mouse:
2 x 100 μmol/kg, p.o.
-20% brain C99
-26% CSF $A\beta_{40}$

Figure 6. Structure of the macrocyclic inhibitor **14**.

300 μmol/kg (~146 mg/kg) with the same PSC833 dosing and sacrifice time as above. Analysis of the brain extracts showed significant amounts of **3** (17.5 μmol/kg) and a robust reduction of $A\beta_{40}$ (−31%) and C_{99} (−46%). The marked effect of the pre-treatment with PSC833 on the efficacy of compound **3** indicated that P-gp mediated efflux is a major factor limiting the brain uptake of the inhibitor and thereby effecting the efficacy of BACE-1 inhibitors.

To overcome the P-gp efflux we modified the P′ site of the inhibitor with another moiety conferring a reduced P-gp susceptibility. With epoxide **13** at hand further variations of the P′ group by epoxide opening with the corresponding amines was straightforward and provided a set of compounds which allowed the identification of inhibitor **14** (Fig. 6).

Tested on the BACE-1 enzyme, **14** displayed an IC_{50} of 2 nM, while an IC_{50} of 24 nM was measured in the cellular system. Interestingly, this macrocycle did not only gain potency towards BACE-1 but also, and to an even greater extent, against human cathepsin D (IC_{50} of 1 nM), thereby losing selectivity. The improved potency in the cell-based assay combined with a slightly reduced P-gp susceptibility (efflux ratio BA/AB = 9.4, MDCK mdr1a) in comparison to **3,** justified in vivo testing of inhibitor **14** by oral application without the co-administration of the P-gp inhibitor PSC833. The compound was given at 100 μmol/kg (~56 mg/kg; sacrifice at 4 h after dosing) and 2 × 100 μmol/kg (0 and 3 h, sacrifice at 7 h) to APP51/16 transgenic mice. The analysis of brain C_{99} showed a significant reduction of −20% ($p = 0.016$) in the double-dosed animals with compound **14** reaching brain levels of 1.5 μmol/kg. The concentration of brain $A\beta_{40}$ was not significantly reduced, but in the CSF a 26% ($p = 0.025$) drop in $A\beta_{40}$ was observed. No effect was found after the single dose when 0.53 μmol/kg of compound **14** were detected in the brain.

In summary, we report the synthesis and in vivo efficacy data of the novel P1–P3 linked macrocyclic BACE-1 inhibitors. The moderately potent macrocycles **2a–c** were improved by exchange of the transition state mimic and variation of the P′ site to the potent and cell active inhibitors **3** (**NB-544**) and **14** (**NB-533**). The binding mode of **3** to BACE-1 was clarified by crystallographic analysis. Both compounds **3** (**NB-544**) and **14** (**NB-533**) showed in vivo activity in a transgenic mouse model, either after double intravenous or after double oral dosing. The in vivo data indicated that C_{99} in forebrain and Aβ in CSF were the most sensitive markers for monitoring the activity of the amyloidogenic pathway of APP in vivo. The efficacious doses were high, and experiments with the P-gp inhibitor PSC833 as well as with mdr knock-out mice (data not shown) identified P-glycoprotein-mediated efflux as one of the major hurdles to reach high brain concentrations.

Acknowledgments

X-ray data collections were performed at the Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland. We are grateful to the machine and beamline groups whose outstanding efforts have made these experiments possible.

We thank C. McCarthy for proofreading the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.055.

References and notes

1. (a) Alzheimer, A. *Allg. Z. Psychiat.* **1907**, *64*, 146; (b) Alzheimer, A. *Cbl. Nervenheil. Psychiat.* **1907**, *30*, 177; (c) Alzheimer, A. *Z. Neurol. Psychiat.* **1911**, *4*, 356–385.
2. Glenner, G. G.; Wong, C. W. *Biochem. Biophys. Res. Comm.* **1984**, *120*, 885.
3. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
4. Haass, C. *EMBO* **2004**, *23*, 483.
5. (a) 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; (b) Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashler, 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; (c) 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.; Zhao, J.; McConlogue, L.; John, V. *Nature* **1999**, *402*, 537; (d) 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; e Lin, X.; Koelsch, G.; Wu, S.; Downs, D; Dashti, A., Tang, J. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1456.
6. Luo, Y.; Bolon, B.; Kahn, S.; Bennett, B. D.; Babu-Khan, S.; Denis, P.; Fan, W.; Kha, H.; Zhang, J.; Gong, Y.; Martin, L.; Louis, J.-C.; Yan, Q.; Richards, W. G.; Citron, M.; Vassar, R. *Nat. Neurosci.* **2001**, *4*, 231.
7. (a) Hannesian, S.; Yun, H.; Hou, Y.; Yang, G.; Bayrakdarian, M.; Therrien, E.; Moitessier, N.; Roggo, S.; Veenstra, S.; Tintelnot-Blomley, M.; Rondeau, J.-M.; Ostermeier, C.; Strauss, A.; Ramage, P.; Paganetti, P.; Neumann, U.; Betschart, C. *J. Med. Chem.* **2005**, *48*, 5175; (b) Machauer, R.; Veenstra, S.; Rondeau, J.-M.; Tintelnot-Blomley, M.; Betschart, C.; Paganetti, P.; Neumann, U. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1361.
8. (a) Ghosh, A. K.; Devasamudram, T.; Hong, L.; DeZutter, C.; Xu, X.; Weerasena, V.; Koelsch, G.; Bilcer, G.; Tang, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 15; (b) Rojo, I.; Martin, J. A.; Broughton, H.; Timm, D.; Erickson, J.; Yang, H.-C.; McCarthy, J. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 191; (c) Stachel, S. J.; Coburn, C. A.; Sankaranarayanan, S.; Price, E. A.; Wu, G.; Crouthamel, M.; Pietrak, B. L.; Huang, Q.; Lineberger, J.; Espeseth, A. S.; Jin, L. *J. Med. Chem.* **2006**, *49*, 6147; (d) Lindsley, S. R.; Moore, K. P.; Rajapakse, H. A.; Selnick, H. G.; Young, M. B.; Zhu, H.; Munshi, S.; Kuo, L.; McGaughey, G. B.; Colussi, D.; Crouthamel, M.-C.; Lai, M.-T.; Pietrak, B.; Price, E. A.; Sankaranarayanan, S.; Simon, A. J.; Seabrook, G. R.; Hazuda, D. J.; Pudvah, N. T.; Hochman, J. H.; Graham, S. L.; Vacca, J. P.; Nantermet, P. G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4057; (e) Moore, K. P.; Zhu, H.; Rajapakse, H. A.; McGaughey, G. B.; Colussi, D.; Price, E. A.; Sankaranarayanan, S.; Simon, A. J.; Pudvah, N. T.; Hochman, J. H.; Allison, T.; Munshi, S. K.; Graham, S. L.; Vacca, J. P.; Nantermet, P. G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5831; (f) Barazza, A.; Götz, M.; Cadamuro, S. A.; Goettig, P.; Willem, M.; Steuber, H.; Kohler, T.; Jestel, A.; Reinemer, P.; Renner, C.; Bode, W.; Mororder, L. *ChemBioChem* **2007**, *8*, 2078.
9. Novo Nordisk, Alcalase 2.5L, type DX, PMN04666.
10. (a) Humphrey, C. E.; Furegati, M.; Laumen, K.; La Vecchia, L.; Leutert, T.; Müller-Hartwieg, C.; Vögtle, M. *Org. Proc. Res.* **2007**, *11*, 1069; (b) Laumen, K.; Ghisalba, O. *Eng. Life Sci.* **2006**, *6*, 193; (c) Laumen, K.; Ghisalba, O.; Auer, K. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1977.
11. Amano, Acylase, ACV12502.
12. Scholl, M.; Trnka, T. M.; Morgan, J. P.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 2247.
13. (a) Barett, A. J. *Biochem. J.* **1970**, *117*, 601; (b) Liaudet-Coopman, E.; Beaujouin, M.; Derocq, D.; Garcia, M.; Glondu-Lassis, M.; Laurent-Matha, V.; Prebois, C.; Rochefort, H.; Vignon, F. *Cancer Lett.* **2006**, *237*, 167.
14. Wang, Q.; Rager, J. D.; Weinstein, K.; Kardos, P. S.; Dobson, G. L.; Li, J.; Hidalgo, I. J. *Int. J. Pharm.* **2005**, *288*, 349.
15. X-ray coordinates for the complex of BACE with inhibitors **2a** and **3** have been deposited in the Protein Data Bank (http://www.rcsb.org), and can be accessed under PDB ID: 3DV1 and 3DV5.
16. Abramowski, D.; Wiederhold, K.-H.; Furrer, U.; Jaton, A.-L.; Neuenschwander, A.; Runser, M. J.; Danner, S.; Reichwald, J.; Ammaturo, D.; Staab, D.; Stoeckli, M.; Rueeger, H.; Neumann, U.; Staufenbiel, M. D. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 411.
17. Gaveriaux, C.; Boesch, D.; Jachez, B.; Bollinger, P.; Payne, T.; Loor, F. *J. Cell. Pharm.* **1991**, *2*, 225.