Bioorganic & Medicinal Chemistry Letters 20 (2010) 603–607

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Macrocyclic BACE-1 inhibitors acutely reduce Aβ in brain after po application

Andreas Lerchner *, Rainer Machauer, Claudia Betschart, Siem Veenstra, Heinrich Rueeger, Clive McCarthy, Marina Tintelnot-Blomley, Anne-Lise Jaton, Sabine Rabe, Sandrine Desrayaud, Albert Enz, Matthias Staufenbiel, Paolo Paganetti, Jean-Michel Rondeau, Ulf Neumann

Novartis Institutes for BioMedicalResearch, Novartis Pharma AG, PO Box, CH 4002, Basel, Switzerland

ARTICLE INFO

Article history:
Received 8 October 2009
Revised 13 November 2009
Accepted 17 November 2009
Available online 22 November 2009

Keywords:
Alzheimer's disease
BACE-1 inhibitors
MDR1–MDCK
P-Glycoprotein
Reduction of Abeta in transgenic APP51/16 mice

ABSTRACT

A series of macrocyclic peptidic BACE-1 inhibitors was designed. While potency on BACE-1 was rather high, the first set of compounds showed poor brain permeation and high efflux in the MDRI–MDCK assay. The replacement of the secondary benzylamino group with a phenylcyclopropylamino group maintained potency on BACE-1, while P-glycoprotein-mediated efflux was significantly reduced and brain permeation improved. Several compounds from this series demonstrated acute reduction of Aβ in human APP-wildtype transgenic (APP51/16) mice after oral administration.

Deposition of amyloid plaques, mainly consisting of 40/42 amino acid β-amyloid peptides, is a pathological hallmark of Alzheimer's Disease (AD). The consecutive action of Beta-site APP Cleaving Enzyme (BACE-1) and γ-secretase generates Aβ peptides from the Amyloid Precursor Protein (APP).[1] They subsequently form oligomers, fibrils and plaques, which are believed to induce neurodegeneration.[2] Mice lacking the gene for BACE-1 are viable and fertile, and do not produce Aβ-peptides. Inhibition of BACE-1 is therefore expected to provide a disease-modifying therapy for AD.[3] BACE-1 is a membrane-bound aspartyl protease that cleaves APP primarily within the acidic endosomes in neurons. BACE-1 binds APP-derived oligo-peptide inhibitors in an extended cleft via multiple hydrogen bond interactions.[4] Nanomolar inhibition of BACE-1 was shown with the peptide inhibitor OM99-2 (MW = 893 Da),[5] but such large and polar compounds do not penetrate the blood–brain barrier (BBB) and therefore lack in vivo activity. In the past decade, synthetic inhibitors of BACE-1 have been reported by a large variety of research groups.[6,7]

Starting from BACE-1 inhibitor **1** (**NB-544**),[8] we aimed to synthesize BACE-1 inhibitors with reduced lipophilicity and better brain penetration (Fig. 1). The core of **1** (ethanolamine and P1–P2 amide group) was retained in series **2**, but the P2–P3 amide group was removed and aromatic rings were inserted into the P1 and P2 positions of the macrocycle. This new scaffold with reduced peptidic character was expected to have better permeability properties than **1**. Previous in vivo studies with macrocyclic BACE-1 inhibitors have shown that the transporter P-glycoprotein (P-gP) in the blood brain barrier considerably limits brain exposure, significant brain levels were achieved either by co-administration of the P-gP-inhibitor PSC833 (Valspodar™), or in P-gP knock-out mice.[8] Similar results were reported by others.[9,10] We aimed to identify potent BACE-1 inhibitors with improved BBB permeability, including limited P-gP mediated efflux. To assess the extent of P-gP efflux on BBB permeability, the well-established in vitro model of Madin Darby canine kidney cells over-expressing the human form of P-gP (MDR1–MDCK) was used.[11] The efflux ratio in this assay is defined as the ratio between the basolateral-to-apical (BL–AP) transfer rate of the molecule across the monolayer relative to its apical-to-basolateral (AP–BL) rate of transfer. In general, compounds with good passive membrane permeability (AP–BL transfer rate) and low efflux ratio tend to exhibit good brain exposure.[12] Compound **3 (NB-216)** was then identified as our first lead compound.

We prepared a series of macrocyclic ethanolamines as depicted in Scheme 1 (according to reaction procedures described in earlier publications).[13,14] 3-Benzylhydroxy-benzaldehyde underwent a Wittig–Horner reaction to provide **4** as a pure *trans*-stereoisomer.

Enantioselective hydrogenation with (*S,S*)-(COD)Et-DuPhos $Rh(I)BF_4$ in EtOAc/MeOH provided the enantiomerically pure amino acid **5** (ee >99%).[15] The choice of the solvents was crucial to provide a good turnover for this reaction. Traces of CH_2Cl_2 or impurities in the starting material were detrimental. Debenzyla-

* Corresponding author. Tel.: +41 616962237; fax: +41 616962455.
E-mail address: andreas.lerchner@novartis.com (A. Lerchner).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.11.092

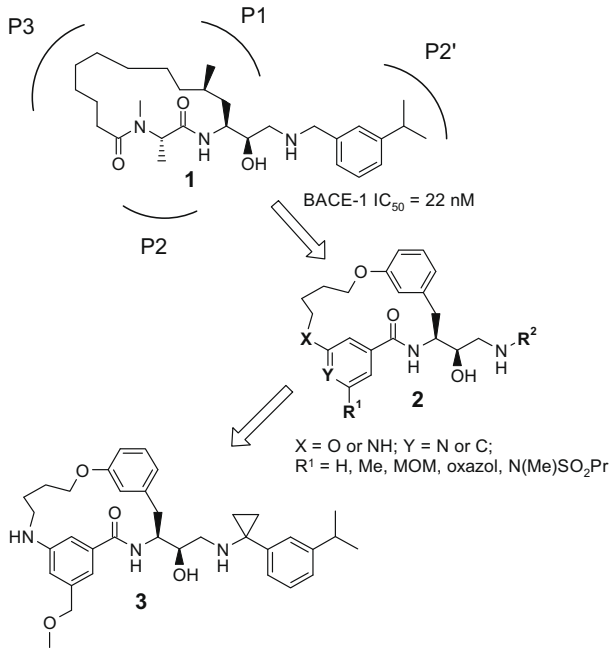


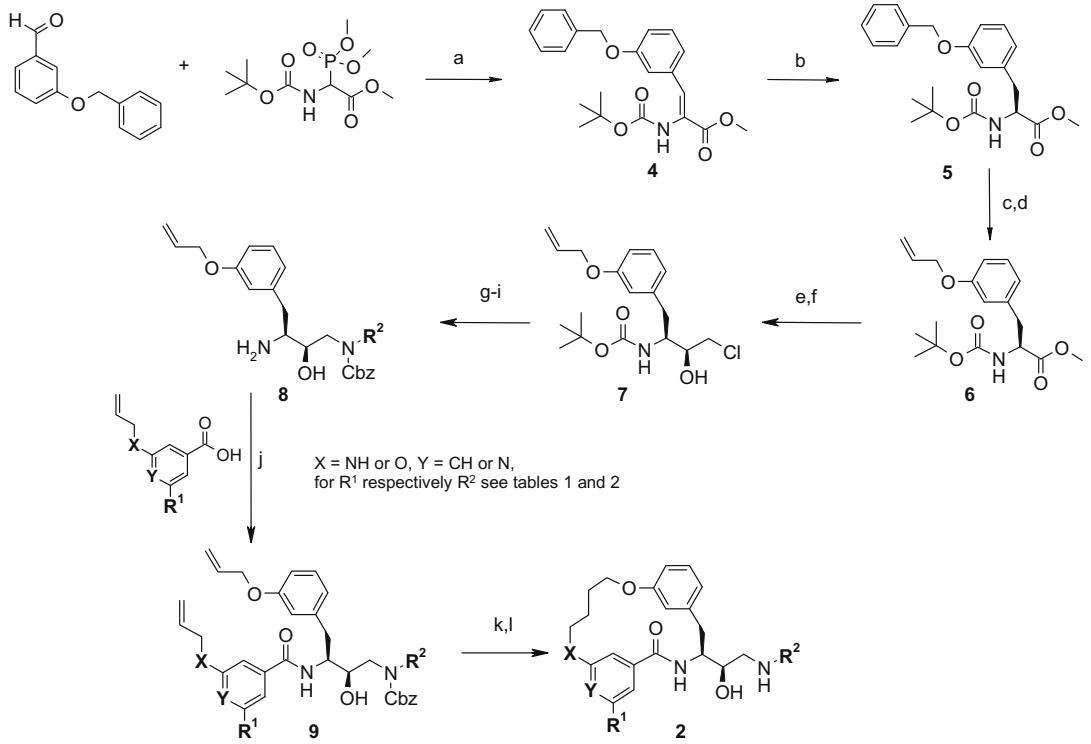
Figure 1. Development of peptidic BACE-1 inhibitor **1** to macrocyclic BACE-1 inhibitor **3**.

tion of **5** and subsequent allylation under standard conditions provided amino acid **6**. Conversion of **6** to **7** was achieved by addition of ICH_2Cl and LDA in THF at −74 °C, followed by reduction with $NaBH_4$ in EtOH at −78 °C; the pure stereoisomer was isolated after purification by column chromatography.[16]

The chlorohydrin **7** was then converted in situ to the epoxide using NaOH in EtOH, and subsequently opened by a series of amines yielding Boc-protected aminoalcohols, which underwent Cbz-protection of the secondary amino group and Boc-deprotection to provide amines **8**. Amide formation of **8** with a series of acids yielded **9**. Ring forming metathesis using the Grubbs II catalyst,[17] followed by Cbz-deprotection and hydrogenation of the formed double bond then provided the desired macrocyclic ethanolamines **2**.

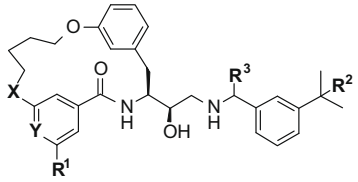
At first, we investigated the SAR of the macrocyclic BACE-1 inhibitors in order to optimize potency (Table 1, compounds **10** to **15**); low nanomolar potency was achieved with compound **15**, where substituent R^1 is propane-1-sulfonic acid methyl-amide. However, testing compounds **10–15** in the MDR1–MDCK assay revealed that they generally had significant efflux (ratio >10) and low passive permeability. It has also been found by others that BACE-1 inhibitors with highly polar P2 substituents are subject of P-gP-mediated efflux.[10]

We then focused on less polar P2 substituents and systematically investigated the environment of the ethanolamino group. Methoxymethyl or methyl groups as substituents R^3 (compounds **16-17**) could not significantly improve the permeability properties, whereas fluorinated alkyl groups (compounds **18-19**) improved the passive permeability and reduced the efflux ratio. Unfortunately, fluorination of the alkyl groups was linked to major reduction of potency on BACE-1. While the mono-fluorinated compound **18** maintained most of the potency in the BACE-1 binding assay, its potency in the cellular assay was weak (IC_{50} in APP over-expressing CHO cells = 2.2 μM).[18] Efforts to further shield the ethanolamino group with sterically more demanding groups led to the investigation of bulkier groups at the benzylic position. Therefore, we investigated dimethylation as well as introduction of cycloalkanes of different ring sizes (Table 2).



Scheme 1. Reagents and conditions: (a) DBU, CH_2Cl_2, 88%; (b) 1% (*S,S*)-(COD)Et-DuPhos Rh(I)BF_4, EtOAc–MeOH, H_2 quant.; (c) Pd/C, H_2, EtOH; (d) K_2CO_3, acetone, allyl bromide, reflux, 95% over two steps; (e) ICH_2Cl, LDA, THF, −74 °C, crude; (f) $NaBH_4$, EtOH, −78 °C, 57% over two steps; (g) H_2NR^2, NaOH, EtOH, 50 °C, 40–60%; (h) CbzCl, CH_2Cl_2, aq Na_2CO_3, quantitative; (i) 4 N HCl in dioxane, quantitative; (j) EDCI, HOBt, NEt_3, CH_2Cl_2, 80–99%; (k) 5% Grubbs II, CH_2Cl_2, 50 °C, 1 h, 30–88%; (l) Pd/C, H_2, THF/EtOH, 60–80%.

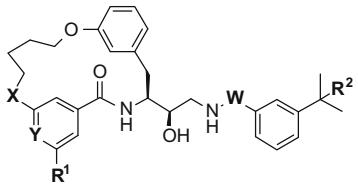
Table 1
SAR for MDR1–MDCK permeation data of macrocyclic BACE-1 inhibitors. BACE-1 activity was measured as described[18]



Compound	X	Y	R^1	R^2	R^3	BACE-1 IC_{50} (nM)	MDR1–MDCK	
							P_{AP-BL} (10^{-6} cm/s)	Efflux ratio P_{BL-AP}/P_{AP-BL}
10	NH	C	H	H	H	292	2.0	17
11	O	N	Me	H	H	42	1.3	23
12	NH	N	Me	H	H	27	0.17	147
13	NH	C	CH_2OCH_3	H	H	60	0.16	174
14	NH	C	1-ox-4-azol-yl	H	H	19	0.11	144
15	NH	C	$N(Me)SO_2Pr$	H	H	1	0.95	16
16	NH	C	CH_2OCH_3	Me	CH_2OCH_3	668	1.3	26
17	O	N	Me	H	Me	83	1.3	24
18	NH	C	CH_2OCH_3	Me	CH_2F	177	3.8	9.6
19	NH	C	CH_2OCH_3	Me	CF_3	>10,000	5.6	3.2

P_{AP-BL} is the permeability trough MDR1-MDCK cell monolayer in the apical-to-basolateral direction, and P_{BL-AP} is the permeability from the basolateral to the apical site of the monolayer, the direction of P-gP-mediated transport.

Table 2
SAR and MDR1–MDCK permeation data of macrocyclic BACE-1 inhibitors; disubstitution in *alpha* position to amino group



Entry	X	Y	R^1	R^2	W	BACE-1 IC_{50} (nM)	MDR1–MDCK	
							P_{AP-BL} (10^{-6} cm/s)	Efflux ratio P_{BL-AP}/P_{AP-BL}
3 (NB-216)	NH	C	CH_2OCH_3	H	Cyclopropyl	17	3.1	4.1
20	O	N	Me	H	Cyclopropyl	17	4.0	3.5
21	O	N	CH_2OCH_3	H	Cyclopropyl	9	4.9	3.7
22	O	C	N-Butyrolactam	H	Cyclopropyl	1	0.4	33
23	NH	C	$N(Me)SO_2Pr$	H	Cyclopropyl	2	0.28	54
24	NH	C	CH_2OCH_3	H	$-CH_2-$	60	0.16	174
25	NH	C	CH_2OCH_3	H	$-CMe_2-$	50	1.8	16
26	NH	C	CH_2OCH_3	Me	Cyclobutyl	67	1.2	10
27	NH	C	CH_2OCH_3	Me	Cyclopentyl	50	1.4	4.4

P_{AP-BL} is the permeability through MDR1–MDCK cell monolayer in the apical-to-basolateral direction, and P_{BL-AP} is the permeability from the basolateral to the apical site of the monolayer, the direction of P-gP-mediated transport.

These substitutions did not generally change inhibitory activity, with cyclopropanes (**3** and **20–23**) showing slightly superior potency. Cyclopropanes were also found to be the best in this series with respect to passive permeability and P-gP efflux. Optimization of potency within the group of cyclopropanes was again possible with more polar substituents R^1 extending into the P2-site, but only at the cost of high efflux and reduced passive permeability (**22** and **23**).

Investigation of compounds **25–27** showed that dimethyl substitution, cyclobutanes and cyclopentanes also led to an improvement in passive permeability and reduction of efflux in comparison to the unsubstituted benzylic amine **24**. However, these changes in permeability properties were inferior to the ones seen with cyclopropane. While it is expected that compounds with a higher molecular weight and higher PSA have lower permeability, as exemplified by modifications of substituent R^1, the effect of the environment of the ethanolamino group on passive permeability and P-gP triggered some more questions.

We observed that the electron-withdrawing substituents fluoroalkyl or cyclopropyl in P1′ improved passive permeability and reduced efflux ratio in the MDR1–MDCK assay (Table 1). Therefore, we assumed that these data could be explained at least partially by basicity changes of the ethanolamine nitrogen in the individual compounds. Measurement of the p*K*a indeed revealed that the poorly permeable compound **17** had a pK_a of 8.5. Compounds **18** and **3,** with moderate permeability had pK_a values of 7.2 and 7.3. Compound **19** with almost no efflux and good permeability had a pK_a of 3.8. Overall, there was a very good correlation between pK_a values of the amino group and permeability properties.

Interestingly, the major impact of the pK_a value was on the efflux ratio, while passive permeability was less affected. On the other hand, significant reduction of basicity decreased the potency on BACE-1 considerably. Compounds with cyclopropyl moieties such as compound **3** (**NB-216**) with an intermediate pK_a of 7.3 seemed to be a good compromise between achieving acceptable brain uptake and maintaining sufficient potency towards BACE-1.

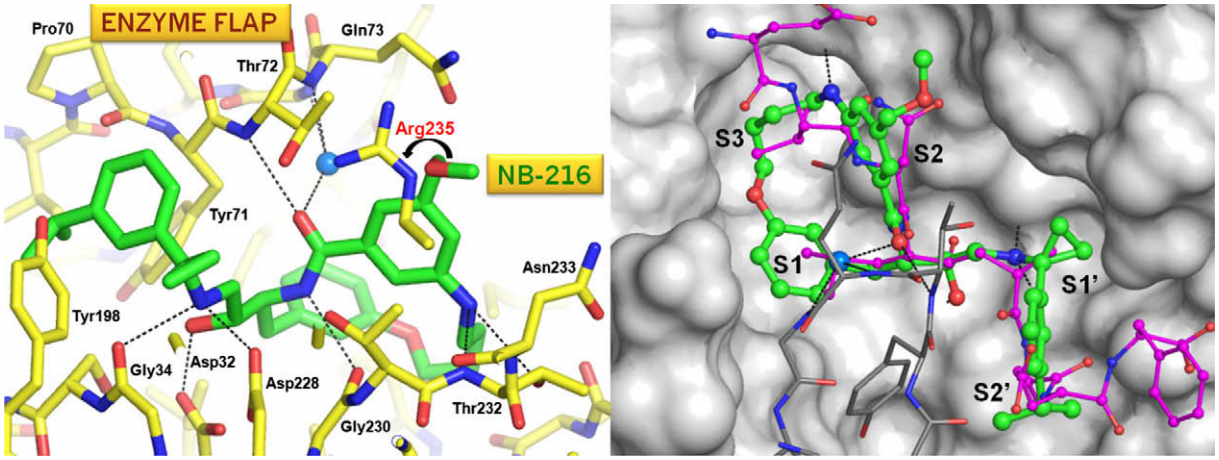


Figure 2. Co-crystal structure of the BACE-1 complex with **3** (NB-216). Left panel: Surface representation showing the **NB-216** complex (green) with the reference inhibitor OM99-2 superimposed (magenta). Right panel. Close-up view of the binding interactions with potential hydrogen-bonded contacts (<3.4 Å) highlighted (dashed lines). Note the polar contact between the R^1 substituent and Arg235.

To gain detailed insights into the binding of compound **3**, the crystal structure of its complex with BACE-1 was determined to 2.1 Å resolution.[19] Close-up views are shown in Figure 2. As illustrated by the structural overlay with OM99-2[4] (Fig. 2, left), **NB-216** spans the S3–S2' enzyme subsites and follows closely the mode of binding of this peptidomimetic inhibitor. In comparison to the linear OM99-2, however, the macrocycle of **NB-216** affords greater conformational rigidity, while filling the contiguous S1–S2–S3 pockets much better, with additional van der Waals contacts to Leu30, Phe108, Ile110, Trp115, Ile118, and residues Gly11, Gln12, and Gly13 of the 10s loop. The R^1 methoxymethylene group is stacked against the Arg235 guanidinium group (Fig. 2, left). This substituent has well-defined electron-density only up to the oxygen atom, indicating that its terminal methyl group remains mobile.

The introduction of the cyclopropyl substituent neither alters the binding of the ethanolamine transition state mimic to the catalytic aspartates, nor the position of the benzyl group in S2' (by comparison with the previously reported X-ray analysis of the BACE-1 complex with **1**[8]).

We selected three cyclopropylamines (compounds **NB-216**, **20**, and **21**) for in vivo investigation and compared them with the unsubstituted benzylamine **11**. After oral administration, drug concentrations in plasma and brain, as well as brain levels of Aβ40 and C99 were measured 4 hours after dosing (Fig. 3, Table 3).[20] The

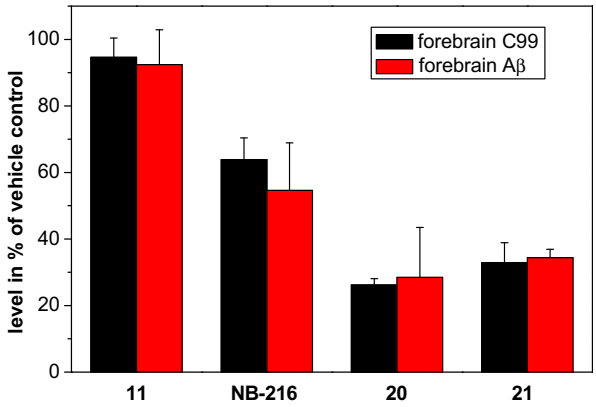


Figure 3. Reduction of Aβ and C99 (levels of forebrain C99 (black bars) and Aβ40 (red bars) relative to vehicle control. Application of compounds po at 60 μmol/kg to APP51/16 tg mice, sacrificed after 4 hours. Vehicle is 2% Cremophor EL in water. Shown are Mean ± SEM.[20]

Table 3
In vitro potency and selectivity, brain and plasma levels, and in vivo efficacy of selected compounds

	11	NB-216	**20**	**21**
IC_{50} BACE-1	32 nM	17 nM	15 nM	9 nM
IC_{50} CHO	55 nM	20 nM	70 nM	47 nM
IC_{50} CathD	156 nM	1 nM	11 nM	3 nM
IC_{50} CathE	8 nM	0.4 nM	4 nM	5 nM
Plasma (μM)	0.03	1.2	3.0	2.2
Brain (μM)	0.04	0.25	0.32	0.54
Aβ40	7%	45%	72%	66%
Significance	p >0.05	p <0.01	p <0.01	p <0.001
C99	5%	36%	74%	67%
Significance	p >0.05	p <0.01	p <0.01	p <0.001

Enzyme and cell assays were done as described.[18] Reductions of Aβ40 and C99 are relative to vehicle control.

three cyclopropanes showed significant concentrations in plasma and brain, in contrast to the low exposure of the unsubstituted benzylic amine **11**. In perfect agreement with brain levels, **11** showed no reduction in Aβ and C99, whereas the tested cyclopropanes demonstrated reduction of Aβ by 45% to 72% and reduction of C99 by 36% to 74%. This study demonstrates that po application of our macrocyclic BACE-1 inhibitors at 60 μmol/kg gave submicromolar brain levels and resulted in acute reduction of Aβ in brain.

It could further be demonstrated that the MDR1–MDCK assay was useful in identifying a chemotype with favorable permeability and limited efflux, which in turn predicted a good brain uptake. The PK/PD data in Table 3 demonstrated a nice correlation of brain concentrations with acute pharmacological effects. Absolute compound brain concentrations were used here, the free fraction in the brain and the concentration in CSF were not determined.

The optimal balance between the four key properties: enzymatic potency, cellular potency, passive permeability and P-gP mediated efflux, was found for compounds with a pK_a of the ethanolamine between 7 and 7.5. Compounds with higher pK_a values usually show high enzymatic and cellular potency, but poor permeability. Lower pK_a values are favorable in terms of permeability and efflux, but we observed a dramatic loss of cellular potency. A similar effect was shown in a recent publication, describing the influence of pK_a modulation on cellular activity, log D values, and oral absorption of ethanolamine BACE-1 inhibitors.[21] Cyclopropylamine type BACE-1 inhibitors have favorable physicochemical properties for in vivo efficacy and are subject of further pharmaco-

kinetic and pharmacological investigations (manuscript in preparation). However, the introduction of the cyclopropyl into ethanolamine type BACE-1 inhibitors led to loss of selectivity over the closely related aspartyl proteases cathepsin D and E. Further investigations are needed to achieve high potency on BACE-1, together with selectivity over cathepsin D and E, and brain permeability.

Acknowledgements

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

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.092.

References and notes

1. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
2. Crouch, P. J.; Harding, S. M.; White, A. R.; Camakaris, J.; Bush, A. I.; Masters, C. L. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 181.
3. Stockley, J. H.; O'Neill, C. *Cell Mol. Life Sci.* **2008**, *65*, 3265.
4. Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. *Science* **2000**, *290*, 150.
5. Ghosh, A. K.; Bilcer, G.; Harwood, C.; Kawahama, R.; Shin, D.; Hussain, K. A.; Hong, L.; Loy, J. A.; Nguyen, C.; Koelsch, G.; Ermolieff, J.; Tang, J. *J. Med. Chem.* **2001**, *44*, 2865.
6. Ghosh, A. K.; Kumaragurubaran, N.; Hong, L.; Kulkarni, S.; Xu, X.; Miller, H. B.; Reddy, D. S.; Weerasena, V.; Turner, R.; Chang, W.; Koelsch, G.; Tang, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1031.
7. Silvestri, R. *Med. Res. Rev.* **2009**, *29*, 295.
8. Machauer, R.; Laumen, K.; Veenstra, S.; Rondeau, J. M.; Tintelnot-Blomley, M.; Betschart, C.; Jaton, A. L.; Desrayaud, S.; Staufenbiel, M.; Rabe, S.; Paganetti, P.; Neumann, U. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1366.
9. Meredith, J. E., Jr.; Thompson, L. A.; Toyn, J. H.; Marcin, L.; Barten, D. M.; Marcinkeviciene, J.; Kopcho, L.; Kim, Y.; Lin, A.; Guss, V.; Burton, C.; Iben, L.; Polson, C.; Cantone, J.; Ford, M.; Drexler, D.; Fiedler, T.; Lentz, K. A.; Grace, J. E., Jr.; Kolb, J.; Corsa, J.; Pierdomenico, M.; Jones, K.; Olson, R. E.; Macor, J. E.; Albright, C. F. *J. Pharmacol. Exp. Ther.* **2008**, *326*, 502.
10. Hussain, I.; Hawkins, J.; Harrison, D.; Hille, C.; Wayne, G.; Cutler, L.; Buck, T.; Walter, D.; Demont, E.; Howes, C.; Naylor, A.; Jeffrey, P.; Gonzalez, M. I.; Dingwall, C.; Michel, A.; Redshaw, S.; Davis, J. B. *J. Neurochem.* **2007**, *100*, 802.
11. Wang, Q.; Rager, J. D.; Weinstein, K.; Kardos, P. S.; Dobson, G. L.; Li, J.; Hidalgo, I. J. *Int. J. Pharm.* **2005**, *288*, 349.
12. Garberg, P.; Ball, M.; Borg, N.; Cecchelli, R.; Fenart, L.; Hurst, R. D.; Lindmark, T.; Mabondzo, A.; Nilsson, J. E.; Raub, T. J.; Stanimirovic, D.; Terasaki, T.; Oberg, J. O.; Osterberg, T. *Toxicol. In Vitro* **2005**, *19*, 299.
13. Betschart, C.; Lerchner, A.; Machauer, R.; Rueeger, H.; Tintelnot-Blomley, M.; Veenstra, S. J. WO Patent 2006074950, 1-13-2006; 20060720, 142.
14. Betschart, C.; Koller, M.; Laumen, K.; Lerchner, A.; Machauer, R.; McCarthy, C.; Tintelnot-Blomley, M.; Veenstra, S. J. WO Patent 2007077004, 12-28-2006; 20070712, 118.
15. Wang, W.; Xiong, C.; Zhang, J.; Hruby, V. J. *Tetrahedron* **2002**, *58*, 3101.
16. Miller, J. F.; Brieger, M.; Furfine, E. S.; Hazen, R. J.; Kaldor, I.; Reynolds, D.; Sherrill, R. G.; Spaltenstein, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3496.
17. Scholl, M.; Trnka, T. M.; Morgan, J. P.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 2247.
18. Machauer, R.; Veenstra, S.; Rondeau, J. M.; Tintelnot-Blomley, M.; Betschart, C.; Neumann, U.; Paganetti, P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1361.
19. X-ray co-ordinates for the complex of BACE-1 with inhibitor NB-216 have been deposited in the Protein Data Bank (http://www.rcsb.org) and can be accessed under code PDB ID: 3K5C.
20. 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. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 411.
21. Charrier, N.; Clarke, B.; Cutler, L.; Demont, E.; Dingwall, C.; Dunsdon, R.; Hawkins, J.; Howes, C.; Hubbard, J.; Hussain, I.; Maile, G.; Matico, R.; Mosley, J.; Naylor, A.; O'Brien, A.; Redshaw, S.; Rowland, P.; Soleil, V.; Smith, K. J.; Sweitzer, S.; Theobald, P.; Vesey, D.; Walter, D. S.; Wayne, G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3674.