Bioorganic & Medicinal Chemistry Letters 21 (2011) 6554–6558

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Aminothiazoles as γ-secretase modulators

Thomas Lübbers [a,*], Alexander Flohr [a], Synese Jolidon [a], Pascale David-Pierson [c], Helmut Jacobsen [b], Laurence Ozmen [b], Karlheinz Baumann [b]

[a] Discovery Chemistry, F. Hoffmann-La Roche Ltd, Grenzacher Strasse 124, 4070 Basel, Switzerland
[b] CNS Discovery, F. Hoffmann-La Roche Ltd, Grenzacher Strasse 124, 4070 Basel, Switzerland
[c] Non-Clinical Safety, F. Hoffmann-La Roche Ltd, Grenzacher Strasse 124, 4070 Basel, Switzerland

ARTICLE INFO

Article history:
Received 22 June 2011
Revised 10 August 2011
Accepted 11 August 2011
Available online 19 August 2011

Keywords:
Alzheimer's disease
γ-Secretase modulator
Amyloid-β peptide
Aβ42 lowering
APPSwe mice model

ABSTRACT

We herein report the discovery of a new γ-secretase modulator class with an aminothiazole core starting from a HTS hit (**3**). Synthesis and SAR of this series are discussed. These novel compounds demonstrate moderate to good in vitro potency in inhibiting amyloid beta (Aβ) peptide production. Overall γ-secretase is not inhibited but the formation of the aggregating, toxic Aβ42 peptide is shifted to smaller non-aggregating Aβ peptides. Compound **15** reduced brain Aβ42 in vivo in APPSwe transgenic mice at 30 mg/kg p.o.

Alzheimer's disease (AD) is the most common dementia with more than 5 million patients alone in the US and 24 million worldwide.[1] It is characterized by progressive memory loss together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. There is currently no cure or prevention for this disease. Brains of AD patients are characterized by the deposition of extracellular neuritic plaques composed out of amyloid beta (Aβ) peptides, by the formation of intracellular neurofibrillary tangles formed by hyperphosphorylated tau proteins and by neuronal loss.[2]

In most cases the disease is sporadic and there is some evidence that the accumulation of Aβ proteins is linked with a reduced clearance of Aβ[3,4] while in familiar AD, gene mutations directly or indirectly increase the production of Aβ42.[5–7] Aβ peptides of different length are formed from the amyloid precursor protein (APP) by successive proteolytic cleavage by BACE and γ-secretase. These secretases are prime targets for a disease modifying approach. γ-Secretase may cleave many different substrates and the inhibition of the enzyme can potentially lead to mechanistically-based side effects. In the case of notch protein processing, a γ-secretase substrate, this became apparent in a recent phase III clinical trial.[8,9] An alternative approach consists in the modulation of γ-secretase where the cleavage of the APP C-terminal fragment is shifted resulting in the formation of more soluble, smaller Aβ peptides such Aβ38 and Aβ37 while the formation of toxic, aggregating Aβ42 is inhibited. γ-Secretase modulators do not inhibit the total activity of the enzyme, and the processing of the other substrates is not affected as demonstrated for notch which is beside APP the other most characterized substrate of γ-secretase.[10–12] It was shown first with a subset of NSAIDs that modulation of γ-secretase is possible.[13] It may lead to a viable approach for drug discovery.[14,15]

In a high through put screen we identified the inosine monophosphate dehydrogenase inhibitor **3** (IMDPH2 IC_{50} = 400 nM) as a moderately potent γ-secretase modulator which is structurally related to the former Phase 1 compound from Eisai, E2012, (**1**)[16–18] and to the Torrey Pines γ-secretase modulator **2**[19] (Fig. 1).

In H4 cells overexpressing human APP[20] the HTS hit **3** lowered Aβ42 and Aβ40 (Aβ42 IC_{50} = 815 nM; Aβ40 IC_{50} = 3700 nM) whereas Aβ38 was increased (Aβ38 EC_{50} = 1500 nM) and total Aβ remained unchanged up to 20 μM. In a cellular notch reporter assay this compound did not show any activity up to the highest concentration of 25 μM tested. In vitro potency may vary substantially when measured in different cellular assays (e.g., Aβ42 IC_{50} of **1** in rat primary neurons = 42 nM).

The synthesis of the modulators exemplified in Scheme 1 proceeded smoothly.

The hydroxy derivate **12** was prepared from the corresponding boc-protected aldehyde **11** via Grignard addition with 4-chlorophenyl magnesium chloride (Scheme 2).

The annelated aminothiazoles were prepared from 2-arylketones **13** via regioselective bromination yielding **14** and subsequent condensation with the thiourea **5a** (Scheme 3, exemplified for compound **15**).

* Corresponding author. Tel.: +41 61 68 83095; fax: +41 61 68 86459.
E-mail address: thomas.luebbers@roche.com (T. Lübbers).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.08.060

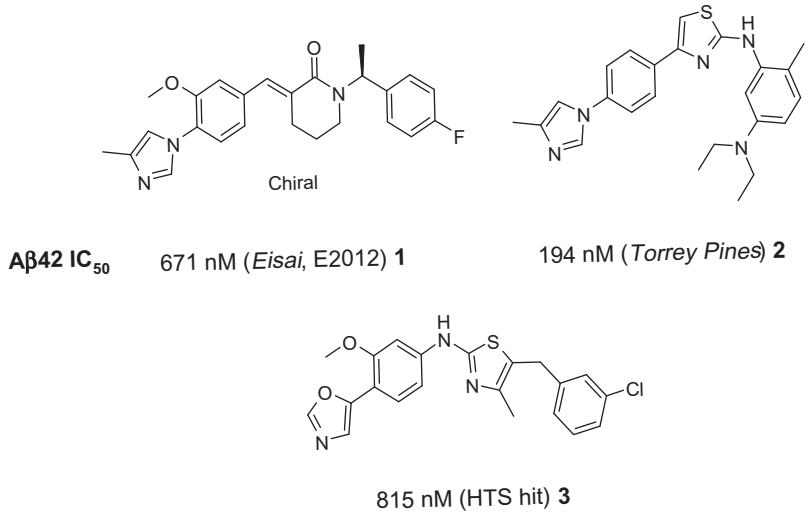
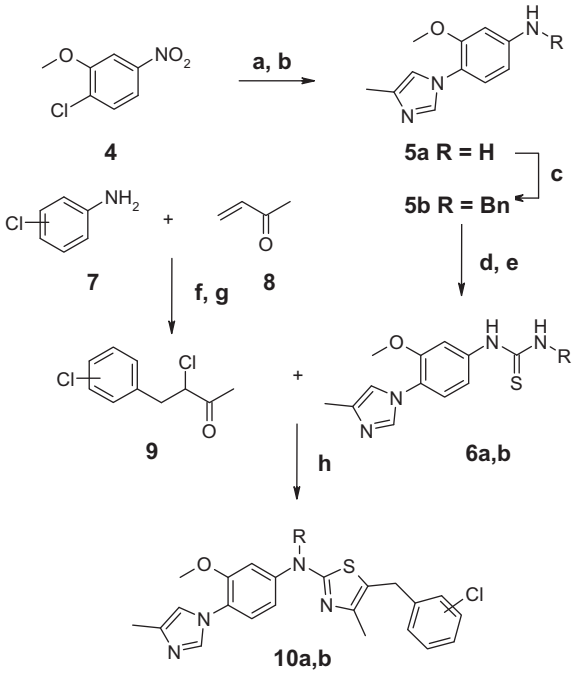
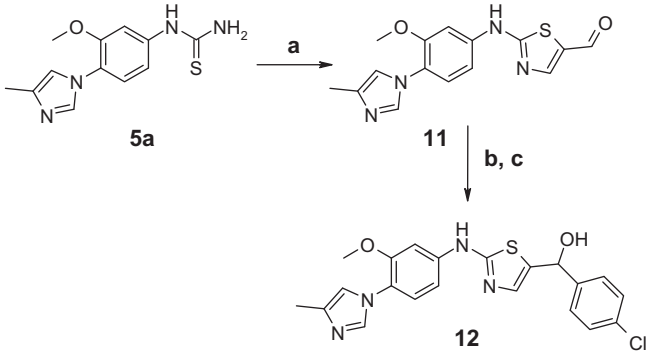


Figure 1. Non-NSAID γ-secretase modulators and the HTS hit (**3**).



Scheme 1. Reagents and conditions: (a) 4-methylimidazole, KOH, DMSO, 80 °C, 5 h, 45%; (b) 1 atm H_2, 10% Pd/C, EtOH, rt, 4 h, 78%; (c) PhCHO, $NaBH_4$, MeOH, rt, 12 h, 85%; (d) PhCONCS, THF, rt, 2 h; (e) K_2CO_3, H_2O, MeOH, 12 h, rt, 82% over two steps; (f) $CuCl_2$, *t*-BuONO, MeCN, rt, 30 min; (g) DBU, Et_2O, rt, 10 min; (h) EtOH, reflux, 12 h, 43–61%.



Scheme 2. Reagents and conditions: (a) $BrCH(CHO)_2$, NaOAc, EtOH, refl., 12 h, 56%; (b) Boc_2O, $DMAP_{cat}$, CH_2Cl_2, 0 °C, rt, 12 h, 49%; (c) (4-Cl-Ph)MgBr, THF, −78 °C, 0 °C, 30 min, 14%.

Different head groups were prepared as shown in the following Schemes 4 and 5 and converted to the final products as demonstrated in the previous synthetic schemes.

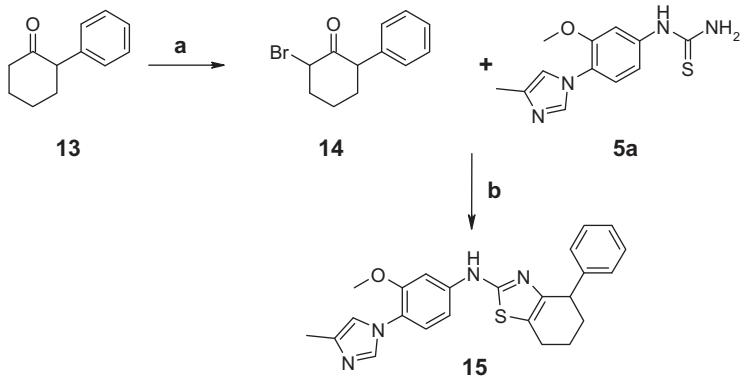
Replacement of the oxazole moiety in the initial HTS hit **3** by the methyl-imidazole group led to a four-fold increase of potency (Table 1, **10a**). Alkylation of the nitrogen linker atom led to loss of potency (**10b**). Introduction of a hydroxyl group at the benzylic position was tolerated (**12**). The position of the benzylic moiety at the thiazole ring seems to be not critical for in vitro potency since **31** was as active as **10a** with the benzyl moiety next to the sulfur atom and not to the nitrogen atom as in **31**.

Compound **10a** was tested in vivo in transgenic APPSwe mice which overexpress human APP with the Swedish FAD mutations[21] and thus led to an increase of Aβ production as compared to wild type mice. **10a** reduced brain Aβ42 by 47% at a dose of 150 mg/kg p.o. after 4 h and by 22% after 7 h. The unfavorable physicochemical properties and the low free fractions of **10a** in mice (below 0.1%) are probably the reason for the only weak in vivo activity.

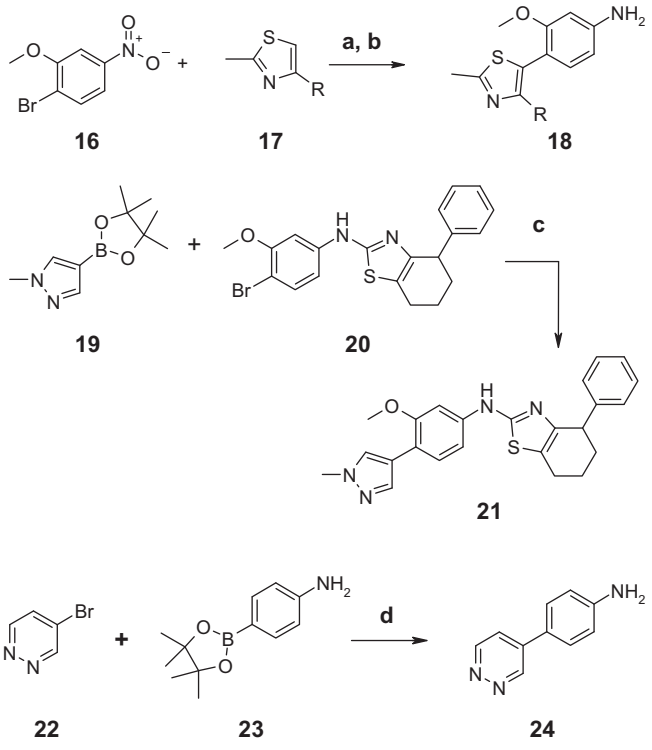
The SAR of the imidazole phenyl head group was investigated with different aryl substituted aminothiazoles (Table 2). In vitro potency was substantially lost when the R^1- and R^2-substituents were missing (**32**). One of the substituents was sufficient to reinstall the in vitro activity (**33** or **34**). The 4-position on the imidazole ring seemed to be critical for CYP inhibition since methylation could strongly reduce this liability (**34** and **35**).

Conformational restriction through annelation of the benzylic position to the aminothiazole ring led to excellent in vitro activity (tetrahydrobenzothiazole derivatives, Table 3). The MeO- and F-derivatives **15** and **37** were in vitro equally potent. The H- and CN-derivatives (**36** and **39**) lost two-fold in vitro activity, all other tested substituents even more.

However, all compounds from this annelated thiazole series were very lipophilic, exhibited low solubilities and had in general a CYP2D6 issue. CYP2D6 IC_{50} values for the MeO-, CN- and Cl-derivatives were below 0.2 μM and for all other derivatives below 0.5 μM. The modulator **15** was tested in a mouse PK experiment (Fig. 2). It had moderate oral bioavailability, moderate volume of distribution and clearance. The brain/plasma ratio was variable but the compound was not a PgP substrate.



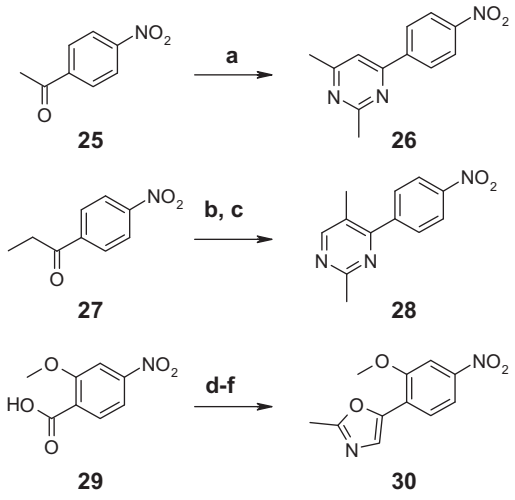
Scheme 3. Reagents and conditions: (a) Br_2, $CHCl_3$, rt, 3 h, crude; (b) EtOH, refl., 1 d, 53%.



Scheme 4. Reagents and conditions: R = H, Me (a) KOAc, $Pd(PPh_3)_{4cat}$, $AcNMe_2$, microwave, 160 °C, 2 h, 42%/62%; (b) $SnCl_2$, EtOH, refl., 1 h, 91%/75%; (c) $Pd(OAc)_2$, KF, MeOH, 140 °C, microwave, 30 min, 16%; (d) $Pd(PPh_3)_{4cat}$, Na_2CO_3, MeCN, H_2O, 3 h, 100 °C, 89%.

We performed a dose response experiment with **15** in the APP-Swe mice model. A good correlation between brain exposure and in vivo efficacy could be established (Fig. 3). The compound exhibited an Aβ42 ED_{50} of roughly 40 mg/kg. At 100 mg/kg p.o. the exposure varied quite substantially probably because of variable absorption of the compound caused by the poor physicochemical properties of **15**.

We found that the imidazole and oxazole head group could be replaced by several other heterocycles. Good in vitro potency was obtained when the nitrogen atom in the head group was either in *meta*-position in the case of five-membered hetaryl rings or in *para*-position for six-membered hetaryl rings. The oxazoles **43**, **49**, **50**, pyrazole **21** and thiazoles **46**, **47** showed good in vitro activity whereas the oxadiazoles **44**, **45** lost in vitro activity. Even the bismethyl pyrimidine **55** retained in vitro activity. The pyridine **53** and pyridazine **54** derivatives exhibited good in vitro potency (Table 4).



Scheme 5. Reagents and conditions: (a) $POCl_3$, MeCN, 90 °C, 2 days, 21%; (b) *t*-$BuO(NMe_2)_2C$, 110 °C, 3 h, quant; (c) $MeC(NH)NH_2{\cdot}HCl$, NaOMe, MeOH, refl., 4 h, 80%; (d) $SOCl_2$, refl., 5 h, quant; (e) $CH_2(CO_2Me_2)_2$, $MgCl_2$, NEt_3, PhMe, rt, 2 h, then aq. HCl_{conc}, rt, 2 h, 49%; (f) $PhI(OAc)_2$, TrifOH, MeCN, refl., 3 h, 56%.

Table 1
Selected SAR around compound **10a**

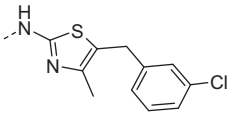
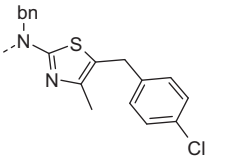
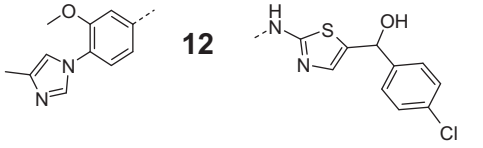
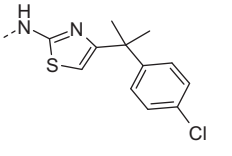
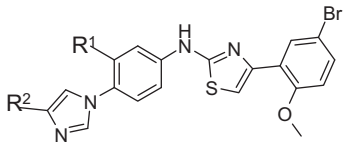
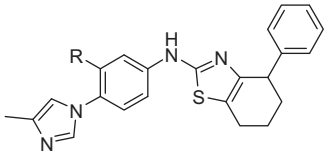
Compound		Aβ42 IC_{50} (nM)
10a		214
10b		>40,000
12		339
31		136

Table 2
SAR around the imidazole phenyl head group



Compound	R^1	R^2	Aβ42 IC_{50} (nM)	CYP_{3A4} IC_{50} (μM)
32	H	H	>5000	<0.2
33	MeO	H	789	<0.2
34	H	Me	880	1.2
35	MeO	Me	898	2.2

Table 3
In vitro activity of annelated thiazoles



Compound	R	Aβ42 IC_{50} (nM)
36	H	133
37	F	52
15	MeO	44
38	HO	209
39	CN	123
40	Me	733
41	Cl	675
42	CF3	>1250

Dose (iv; po) [mg/kg]	0.7, 7.2
Clp [mL/min/kg]	18.3
Vd_{ss} [L/Kg]	4.6
t½ (po) [h]	2.5
F [%]	25
AUC_N0-24 po [μM h kg/mg]	231
Brain/plasma	0.4-2.1

Figure 2. Pharmacokinetic profile of **15** in mice.

The pyrazole derivative **21** had a low plasma exposure and was in vivo inactive in the APPSwe mice model (100 mg/kg p.o. 4 h). In contrast the methyl oxazole derivative **43** showed a 47% reduction of brain Aβ42 in the same model at 100 mg/kg p.o. The plasma exposure was in the same range as for the methyl imidazole **15** but the brain/plasma ratio was lower (0.34). The plasma exposure of the mono-methyl thiadiazole **46** and of the pyridine derivative **53** were low and therefore these compounds were inactive in vivo at 100 mg/kg p.o. The pyridine **53** and the pyridazine derivatives **54** inhibited strongly the CYP2D6 enzyme.

In summary, we explored the SAR around a γ-secretase HTS hit (**3**). We could improve in vitro activity of the open-chain γ-secretase modulators substantially when we fixed the conformation of the benzyl ring through annelation to the aminothiazole ring. The γ-secretase modulator **15** was in vivo active at 30 mg/kg. Replacement of the imidazole head group by different heteroaryl moieties did not provide superior compounds in respect to their in vitro or in vivo potency.

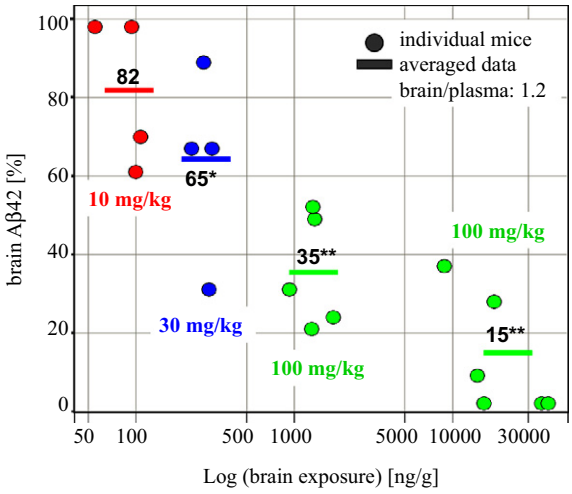
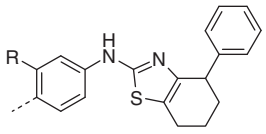
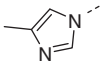
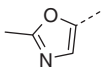
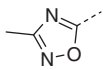
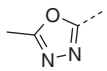
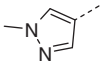
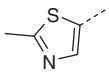
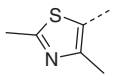
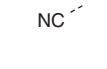
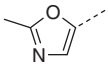
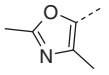
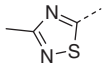
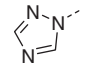
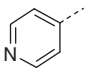
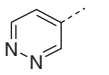
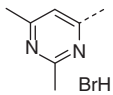
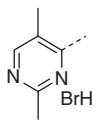


Figure 3. correlation of brain Aβ42 levels with brain exposures of **15** in APPSwe mice in vivo (p.o. after 4 h).

Table 4
Influence of the head group on the in vitro potency



R	Aβ42 IC_{50} (nM) (compound)			
MeO	 44 (**15**)	 86 (**43**)	 3431 (**44**)	 999 (**45**)
	 244 (**21**)	 252 (**46**)	 212 (**47**)	 3358 (**48**)
H	 133 (**49**)	 127 (**50**)	 651 (**51**)	 5548 (**52**)
	 319 (**53**)	 376 (**54**)	 483 (**55**)	 2868 (**56**)

Acknowledgments

We thank S. Herzig, N. Schinkel, N. Benedetti, M. Mayer, T. Meier, M. Bender and R. Schubenel for their dedicated technical assistance.

References and notes

1. Jellinger, K. A. *J. Neural Transm.* **2006**, *113*, 1603.
2. Finder, V. H. *J. Alzheimer Dis.* **2010**, *22*, S5.
3. Alvira-Botero, X.; Carro, E. M. *Curr. Aging Sci.* **2010**, *3*, 219.
4. Mawuenyega, K. G.; Sigurdson, W.; Ovod, V.; Munsell, L.; Kasten, T.; Morris, J. C.; Yarasheski, K. E.; Bateman, R. J. *Science* **2010**, *330*, 1774.
5. Scheuner, D.; Eckman, C.; Jensen, M., et al *Nat. Med.* **1996**, *2*, 864.

6. Borchelt, D. R.; Thinakaran, G.; Eckman, C. B., et al *Neurons* **1996**, *17*, 1005.
7. Duff, K.; Eckman, C.; Zehr, C., et al *Nature* **1996**, *383*, 710.
8. Saxena, U. *Expert Opin. Ther. Targets* **2010**, *14*, 273.
9. Imbimbo, B. P.; Panza, F.; Frisardi, V.; Solfrizzi, V.; D'Onofrio, G.; Logroscino, G.; Seripa, D.; Pilotto, A. *Expert Opin. Investig. Drugs* **2011**, *20*, 325.
10. De Strooper, B. et al *Nature* **1999**, *398*, 518.
11. Douglas, W. E. *Neuroscientist* **2010**, *16*, 614.
12. Golde, T. E. et al *Science* **2009**, *324*, 603.
13. Weggen, S.; Eriksen, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Pietrizik, C. U.; Findlay, K. A.; Smith, T. E.; Murphy, M. P.; Bulter, T.; Kang, D. E.; Marquez-sterling, N.; Golde, T. E.; Koo, E. H. *Nature* **2001**, *414*, 212.
14. Review Oehlrich, D.; Berthelot, D. J.-C.; Gijsen, H. J. M. *J. Med. Chem* **2011**, *54*, 669.
15. Review Pettersson, M.; Kauffman, G. W.; am Ende, C. W.; Patel, N. C.; Stiff, C.; Tran, T. P.; Johnson, D. S. *Expert Opin. Ther. Patents* **2011**, *21*, 205.
16. Portelius, E.; Van Broeck, B.; Andreasson, U.; Gustavsson, M. K.; Mercken, M.; Zetterberg, H.; Borghys, H.; Blennow, K. *J. Alzheimer Dis.* **2010**, *21*, 1005.
17. Nagy, C.; Schuck, E.; Ishibashi, A.; Nakatani, Y.; Rege, B.; Logovinsky V. Eisai Inc., 10th International Conference on Alzheimer's & Parkinson's Diseases, March 9–13, 2011, Barcelona, Spain Poster Session 2. Abstract# 839.
18. Hashimoto, T.; Ishibashi, A.; Hagiwara, H.; Murata, Y.; Takenaka, O.; Miyagawa T. 13th International Conference on Alzheimer's Disease (ICAD 2010), July 10–15, 2010, Honolulu, Hawaii, Abstract No. P3–307.
19. Kounnas, M. Z.; Danks, A. M.; Cheng, S.; Tyree, C.; Ackerman, E.; Zhang, X.; Ahn, K.; Nguyen, P.; Comer, D.; Mao, L.; Yu, C.; Pleynet, D.; Digregorio, P. J.; Velicelebi, G.; Stauderman, K. A.; Comer, W. T.; Mobley, W. C.; Li, Y.-M.; Sisodia, S. S.; Tanzi, R. E.; Wagner, S. L. *Neuron* **2010**, *67*, 769.
20. *Cellular γ-secretase assay*: Human neuroglioma H4 cells overexpressing human APP were plated at 30,000 cells/well/200 μl in 96-well plates in IMDM media containing 10% FCS, 0.2 mg/l Hygromycin B and incubated for 2 h at 37 °C, 5% CO_2 prior to adding test compounds. Compounds for testing were dissolved in 100% Me_2SO yielding in a 10 mM stock solution. Typically 12 μl of these solutions were further diluted in 1000 μl of IMDM media (w/o FCS). Sub sequential 1:1 dilutions gave a ten point dose response curve. Hundred microliter of each dilution was added to the cells in 96-well plates. Appropriate controls using vehicle only and reference compound were applied to this assay. The final concentration of Me_2SO was 0.4%. After incubation for 22 h at 37 °C, 5% CO_2, 50 μl supernatant was transferred into round-bottom 96-well polypropylene plates for detection of Aβ42. Fifty microliter assay buffer (50 mM Tris/HCl, pH 7.4, 60 mM NaCl, 0.5% BSA, 1% TWEEN 20) was added to the wells followed by the addition of 100 μl of detection antibody (ruthenylated Aβ42-specific antibody BAP15 0.0625 μg/mL in assay buffer). 50 μl of a premix of capture antibody (biotinylated 6E10 antibody, 1 μg/mL) and Steptavidin-coated magnetic beads (Dynal M-280, 0.125 mg/mL) were preincubated for 1 h at room temperature before adding the assay plates. Assay plates were incubated on a shaker for 3 h at room temperature and finally read in the Bioveris M8 Analyser according to the manufacturer's instructions (Bioveris).
21. *APP Swe mice model*: Selected compounds were administered p.o. to ca. 3 month old transgenic mice overexpressing APP-Swe under the control of mouse Thy-1 promoter. After 4 h brain homogenates (1% DEA) were measured for Aβ38, 40, 42 and total Aβ by LP ECL. For PK and PD studies compound levels were measured in brain and plasma using LC–MS/MS analytics.