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Aminoimidazoles as BACE-1 inhibitors: The challenge to achieve in vivo brain efficacy

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ABSTRACT

The evaluation of a series of bicyclic aminoimidazoles as potent BACE-1 inhibitors is described. The crystal structures of compounds **14** and **23** in complex with BACE-1 reveal hydrogen bond interactions with the protein important for achieving potent inhibition. The optimization of permeability and efflux properties of the compounds is discussed as well as the importance of these properties for attaining in vivo brain efficacy. Compound (*R*)-**25** was selected for evaluation in vivo in wild type mice and 1.5 h after oral co-administration of 300 μmol/kg (*R*)-**25** and efflux inhibitor GF120918 the brain Aβ40 level was reduced by 17% and the plasma Aβ40 level by 76%.

Alzheimer's disease (AD) is a neurodegenerative brain disorder characterized clinically by progressive decline of cognitive function. Pathologically, AD is characterized by amyloid plaques,[1] containing Aβ peptide(s), and by neurofibrillary tangles (NFTs) containing hyper-phosphorylated tau protein. Aβ peptides are produced from membrane-bound β-amyloid precursor protein (APP) by the sequential proteolytic cleavage of two aspartyl proteases, β- and γ-secretase. β-Secretase (β-site APP cleaving enzyme, BACE-1), has been identified as the enzyme responsible for the initial processing of APP.[2] Processes that limit the accumulation of neurotoxic Aβ peptides could offer effective treatments of AD. Thus inhibition of BACE-1 represents a strategy for the development of disease-modifying therapeutics for the treatment of AD.[3]

The original BACE-1 inhibitor lead dihydroisocytosine **1**, emanating from fragment based lead generation,[4] was the starting point for scaffold hopping via the aminohydantoin **2** into the bicyclic aminoimidazoles **3** as depicted in Figure 1. One of the reasons for selecting the bicyclic lead **3** was the possibility to fine-tune the properties of the amidine moiety by introducing R substituents on the bicyclic ring of **3**. Thus independently from others[5] we embarked on investigating this compound class. In this paper we will discuss the properties important for in vivo brain efficacy and describe the effort to improve bicyclic aminoimidazole derivatives **3** towards achieving in vivo brain efficacy. We disclose novel BACE-1 inhibitors with enhanced permeability properties, culminating in the design of R-(**25**) displaying Aβ40 lowering effects in mice brain.

Within the first scoping activities of this series different aromatic rings as well as aromatic ring substituents were evaluated for potency and ADME properties.

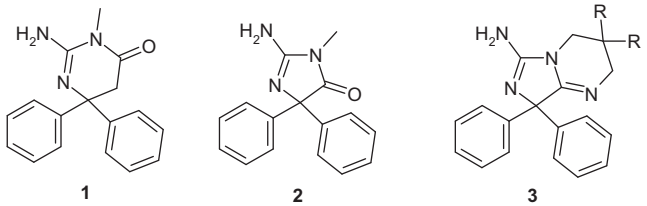

Figure 1. Scaffold hopping from dihydroisocytosines to aminohydantoins to aminoimidazoles.

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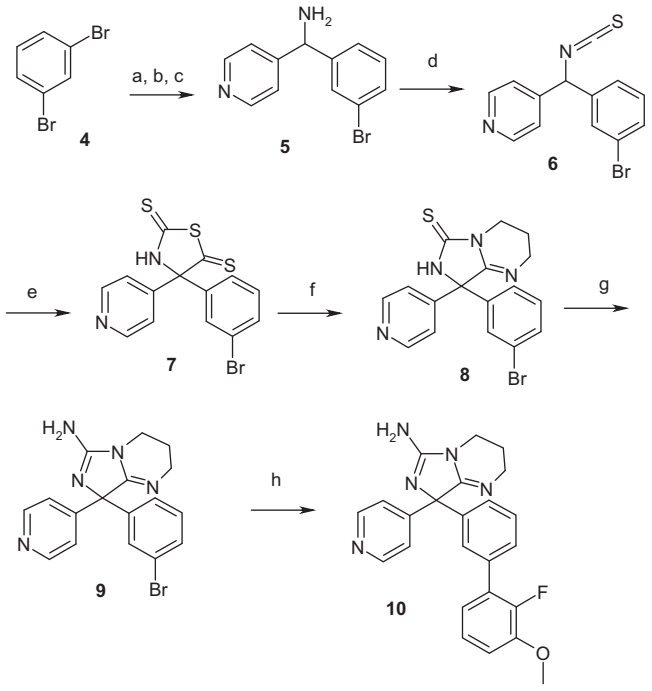


Scheme 1. Reagents and conditions: (a) BuLi, THF, −78 °C; (b) 4-cyanopyridine; (c) $NaBH_4$, MeOH, rt, 12 h, 62%; (d) $(im)_2CS$, CH_2Cl_2, ~100%; (e) CS_2, *t*-BuOK, THF, −78 to 0 °C, ~100%; (f) propylenediamine, EtOH, 80 °C, 2 h, 89%; (g) TBHP, NH_4OH, MeOH, 40 °C, 12 h, ~100%; (h) 2-fluoro-3-methoxyphenyl boronic acid, $Pd(dppf)Cl_2$, $CsCO_3$, DME–H_2O–EtOH 6:3:1, MW 130 °C, 45%.

The pyridine containing tetrahydroimidazopyrimidine analogues[6] were synthesized as exemplified in Scheme 1. The procedure was modified especially in the first step, compared to the published synthesis,[5] to allow for the introduction of pyridine. 1,3-Dibromobenzene **4** was treated with 1 equiv of butyl lithium in THF at −78 °C, followed by reaction with 4-cyanopyridine to give the imine which was reduced to amine **5** by reaction with sodium borohydride in methanol at room temperature over night. Treatment of the amine with thiocarbonyldiimidazole in dichloromethane quantitatively gave the isothiocyanate **6** which was reacted with potassium *tert*-butoxide and carbon disulfide in THF at low temperature to give the thiazolidine-2,5-dithione derivative **7**. Reaction with propylenediamine gave 3,4,7,8-tetrahydroimidazo[1,5-*a*]pyrimidine-6-thione derivative **8** which was treated with ammonia and *tert*-butylhydroperoxide in methanol to give the 2,3,4,8-tetrahydroimidazo[1,5-*a*]pyrimidine-6-amine (THIP) derivative **9**. In the final step, the 2-fluoro-3-methoxyphenyl group was introduced by a palladium catalyzed microwave assisted Suzuki[7] reaction to give compound **10**.

The in vitro inhibition of BACE-1 was determined using a fluorescence energy transfer FRET-based screen.[8] The pIC_{50} values and the Caco-2 permeability[9] values for selected compounds are shown in Table 1. Potent BACE-1 inhibition can be attained in this series, as for example, shown for compound **13**, but low Caco-2 permeability values as exemplified for **10–12** were limiting their use as pharmacological probes. High Caco-2 values (>10) are an indicator for good blood brain permeability properties and an improvement for this series was needed.

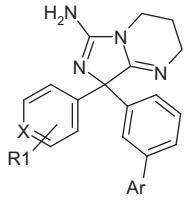
The polar surface area (PSA) of the compounds was in an acceptable range for passing the blood brain barrier (BBB), so we reasoned that the low permeability could be due to the high basicity of the aminoimidazole group (pK_a estimated to be >8) and that it is the fraction of non-protonated species that permeates the membrane. To increase the amount of the neutral form a reduction of the pK_a seemed reasonable. Therefore, a di-F moiety was introduced in the bicyclic ring to allow for a lowering of the pK_a. The synthesis of these compounds followed the same procedure as described for **10**, but the diamine in the ring forming reaction (Scheme 1, step f) was replaced with 2-di-F-propane-1,3-diamine.[10]

The di-F substituted tetrahydroimidazopyrimidines generally displayed increased Caco-2 permeability together with decreased pK_a[11] as shown in Table 2. In some instances, as for **15** and **16**, the permeability was still low and apparently other factors than pK_a were also contributing to the compounds permeability properties. We suspected that transporters could be involved and therefore we started to assess efflux in the Caco-2 assay.[9] The measured efflux values as shown in Table 2 also confirmed that the compounds are substrates for transporters.

The compounds were also assessed for their ability to inhibit the formation of sAPPβ in a cell-based assay.[12] There was generally a good correlation between the pIC_{50} values in the FRET and the cell assay, except when the pK_a of the compounds were below ~6. In these cases, a drop-off in potency in the cell assay could be observed, probably due to the smaller fraction of inhibitors being protonated by the catalytic aspartates. A pK_a below 6 was measured or predicted for pyridines as in examples **14**, **15** and **20**. Both **15** and **20** display a drop-off that can be explained by pK_a but the lack of drop-off for **14** is not fully understood.

One of the more potent analogues (**14**) was subjected to crystallization in BACE-1 protein and the structure of the complex was determined at 1.75 Å resolution (Fig. 2).[13] The aminoimidazole moiety of compound **14** interacts via a hydrogen bond network to the two catalytic residues Asp32 and Asp228, as previously shown for BACE-1 inhibitors containing an aminoheterocycle moiety.[4] We hypothesize that a proton is shared between Asp32 and the aminoimidazole resulting in a formal charge of −1 for the catalytic residues and the compound together. This would be the same charge state as in the catalytically active enzyme–substrate complex where one of the aspartic residues is believed to be protonated and the peptide bond together with the nucleophilic water is neutral.[14] However, it cannot be excluded that an additional proton is shared between Asp32 and Asp228 in this complex as the closest carboxyl oxygens are only 3.1 Å apart. Compound **14** binds to a protein conformation where the so called flap is open. This allows the R1 substituted aryl to interact with Trp76. In the case of **14** the nitrogen of the 4-pyridyl ring accepts a hydrogen bond from Trp76. The di-F substitution on the tetrahydroimidazopyrimidine is completely solvated and does not interfere with any parts of the protein.

Table 1
Biological activities of tetrahydroimidazopyrimidines

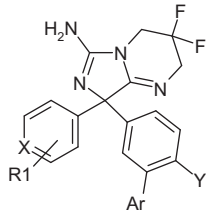


	X	R1	Ar
10	N	H	2-F, 3-OCH_3-Ph
11	N	H	5-pyrimidinyl
12	C	4-OCH_3	5-pyrimidinyl
13	C	4-OCH_3	3,5-di-Cl-Ph

Compound	pIC_{50}[a]	Caco-2 (10^{-6} cm/s)	pK_a	PSA (Å)
10	7.05	1.0	nd	68
11	6.58	0.6	7.6	79
12[5]	7.04	0.6	nd	78
13	7.65	nd	nd	57

nd; not determined.
[a] Values are means of $n \geq 2$ determinations, absolute value of standard deviation ≤10%.

Table 2
Biological activities of di-F-tetrahydroimidazopyrimidines



	X	Y	R1	Ar
14	N	H	H	2-F, 3-OCH_3-Ph
15	N	H	H	5-pyrimidinyl
16	C	H	4-OCH_3	5-pyrimidinyl
17	C	H	4-$OCHF_2$	5-pyrimidinyl
18	C	H	4-$OCHF_2$	5-OCH_3, 3-pyridinyl
19	C	H	4-$OCHF_2$	2-F, 3-pyridinyl
20	N	H	H	2-F, 5-OCH_3-Ph
21	C	F	4-OCH_3 ,3-CH_3	3-pyridinyl

Compound	pIC_{50}[a]	pIC_{50} cell[a] sAPPβ	Caco-2 (10^{-6} cm/s)	Efflux ratio	pK_a
14	7.39	7.27	7.0	7.7	nd
(*R*)-**14**	7.69	7.51	6.7	8.1	5.3
15	6.97	5.92	0.6	nd	nd
16	7.00	6.74	1.1	nd	6.8
17	7.63	7.71	4.1	nd	6.1
18	7.86	7.85	2.4	7.5	nd
19	7.52	7.90	6.6	nd	nd
20	7.54	6.55	8.0	6.3	5.9
21	7.43	7.35	3.3	nd	nd

nd; not determined.
[a] Values are means of $n \geq 2$ determinations, absolute value of standard deviation ≤10%.

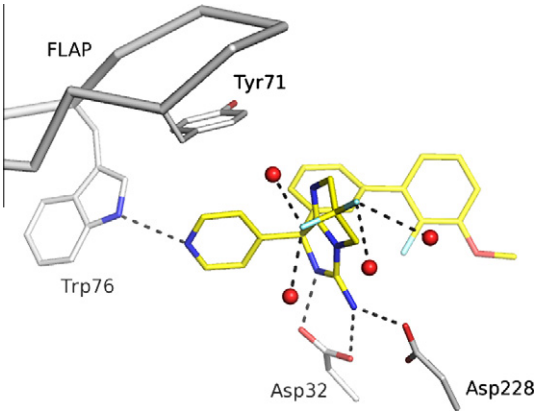
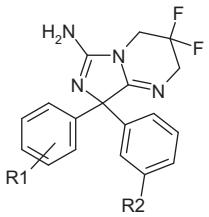


Figure 2. Crystal structure of compound **14** bound to BACE-1, PDB code 4acu. Key interactions between inhibitor (yellow), protein (gray), N (blue), O (red) and water molecules (red balls) are highlighted with dashed lines. Figures made using PyMOL.[15]

The difluoromethoxy substituent as in analogue **17**, compared to methoxy **16** and pyridine **15**, increased the potency both in the FRET and in the cell-based assay. A similar trend was observed in compounds **18** and **19**, both displaying good potencies. Compound **20** with the highest permeability displayed a drop-off in potency when going from the FRET to the cell assay. We also investigated if an electron withdrawing substituent such as fluoro in the aryl ring examplified by **21** could have a positive effect on the permeability. This effect was minor and the permeability was equal for Ar such as **16** and best permeability was demonstrated by compound **21**.

When examining some Br-intermediates, we found that a compound without the biaryl motif as in **22** (Table 3) showed better permeability and efflux properties when compared to the corresponding biaryl analogues. We decided to explore this further by introducing aryl group replacements and a few examples are shown in Table 3. The alkynes **23** and **24** were potent BACE-1 inhibitors in the cell assay and the F-propyl ether analogue **25**

Table 3
Biological activities of non-biaryl di-F-tetrahydro-imidazopyrimidines



	R1	R2
22	4-OCH_3	Br
23	4-$OCHF_2$	$CCCH_2OCH_3$
24	4-$OCHF_2$	$CCCH_2CH_2OCH_3$
25	4-$OCHF_2$	$OCH_2CH_2CH_2F$

Compound	pIC_{50}[a]	pIC_{50} cell[a] sAPPβ	Caco-2 (10^{-6} cm/s)	Efflux ratio	pK_a
22	5.47	nd	11	1.2	nd
23	7.11	7.46	8.4	3.5	nd
24	7.25	7.87	9.5	3.6	nd
25	7.23	7.83	11	3.2	6.5
(*R*)-**25**	7.50	8.10	8	0.8	nd

nd; not determined.
[a] Values are means of $n \geq 2$ determinations, absolute value of standard deviation ≤10%.

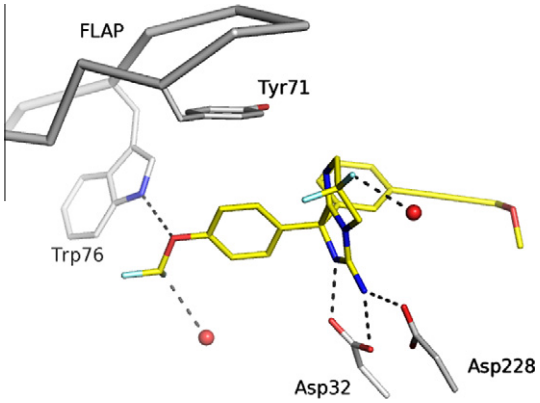


Figure 3. Crystal structure of compound **23** in complex with BACE-1, PDB code 4acx. Key interactions between inhibitor (yellow), protein (gray), N (blue), O (red) and water molecules (red balls) are highlighted with dashed lines.

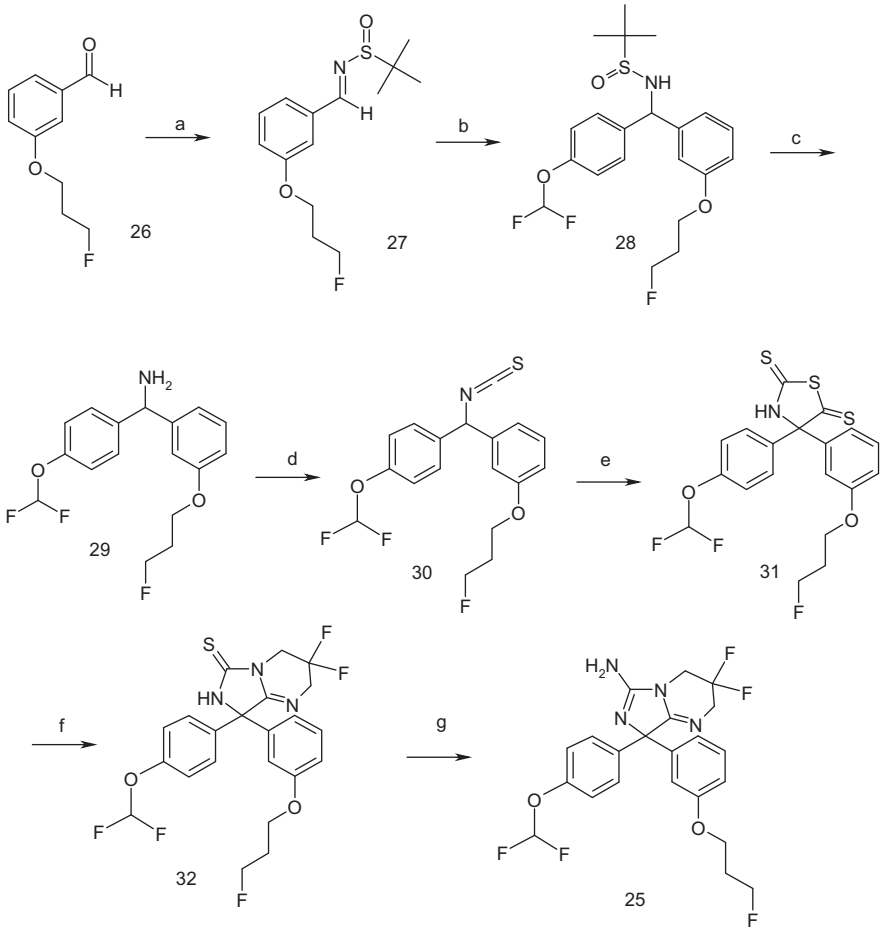
displaying the best permeability value was selected for enantiomeric HPLC separation. The more active enantiomer of **25**, (*R*)-**25**, showed a better efflux ratio than the racemate.

Alkynes **23** and **24** were synthesized using the general method as described in Scheme 1, but the last reaction (Scheme 1, step h) was replaced by a Pd catalysed coupling of the bromides using the Sonogashira protocol and the appropriately substituted acetylenes.[16]

The crystal structure of compound **23** in complex with BACE-1 has been refined to 2.0 Å resolution and is shown in Figure 3.[13] Compound **23** overlaps well with the structure of compound **14**. However, in this case the oxygen of the R1 substituent forms the hydrogen bond interaction with Trp76. The R2 alkyne extends towards the S3 pocket.

Compound **25** was synthesized following a somewhat modified procedure, compared to Scheme 1, starting from the F-propyloxy substituted benzaldehyde **26** as shown in Scheme 2. The aldehyde was converted into the sulfinimine **27** which was subsequently treated with a modified Grignard reagent made from 1-bromo-4-difluoromethoxybenzene to yield derivative **28**. The sulfinamide was then hydrolyzed to amine **29** with HCl in Et_2O. Treatment of the amine with thiophosgene in dichloromethane quantitatively gave the isothiocyanate **30** which was reacted with potassium *tert*-butoxide and carbon disulfide in THF at low temperature to give the thiazolidine-2,5-dithione derivative **31**. Reaction with 2.2-difluoropropane-1,3-diamine at elevated temperature over night yielded compound **32** which was treated with ammonia and *tert*-butylhydroperoxide in methanol to give the desired di-F-tetrahydroimidazopyrimidine derivative **25**.

The F-propyl ether (*R*)-**25** being a low nM inhibitor of BACE-1 in the cell assay, displaying reasonable permeability and efflux ratio, was selected for in vivo studies. The plasma and brain concentrations of (*R*)-**25** were examined and it was found that the compound had a low brain/plasma ratio as shown in Figure 4. However, when a Pgp/BCRP inhibitor (GF120918)[17] was dosed before (*R*)-**25** the



Scheme 2. Reagents and conditions: (a) 2-methyl-2-propanesulfinamide, Ti(IV)ethoxide, THF, 78%; (b) (1) *n*-BuLi (2.5 M in hexane), *i*PrMgBr (1 M in THF), THF, 0 °C; (2) 1-bromo-4-(difluoromethoxy)benzene, THF, −65 °C, 97%; (c) HCl (1.0 M in Et_2O), MeOH, 12 h, 45%; (d) Cl_2CS, Na_2CO_3 (satd), CH_2Cl_2, 0 °C, ~100%; (e) CS_2, *t*-BuOK, THF, −78 °C, ~100%; (f) 2,2-difluoropropane-1,3-diamine dihydrochloride, *N*,*N*-diisopropylethylamine, EtOH, 70 °C, 55%; (g) TBHP, NH_4OH, MeOH, 12 h, 46%.

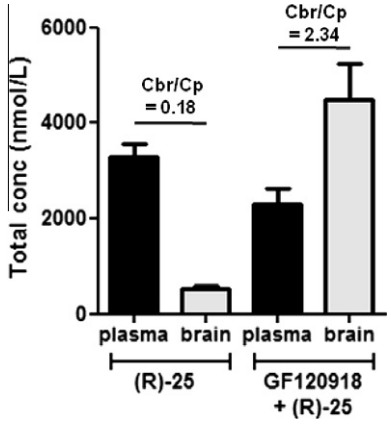


Figure 4. In vivo plasma and brain concentration in C57BL/6 mice of compound (*R*)-**25** compared to plasma and brain concentration with pre-dosed Pgp/BCRP inhibitor GF120918.

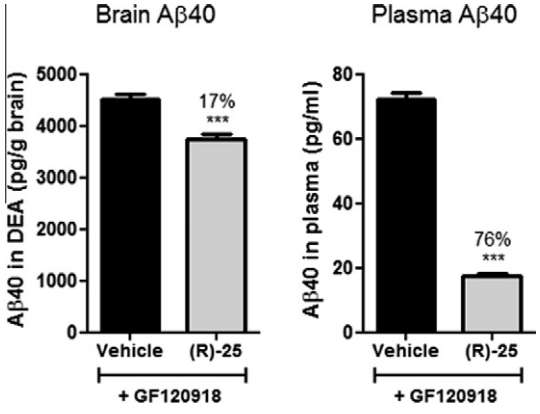


Figure 5. Reduction of Ab40 levels in brain and plasma of C57BL/6 mice by compound (*R*)-**25** dosed together with Pgp/BCRP inhibitor GF120918.

brain/plasma ratio was increased considerably from 0.18 to 2.3.[18] Even though the compound displayed minor efflux in Caco-2 cells this did not fully reflect the efflux properties of transporters expressed at the BBB, since inhibiting Pgp/BCRP increased the Cbr/Cpl ratios >10×. Apparently the effect of transporters like Pgp can not be fully evaluated in Caco-2 cells and the efflux ratio of (*R*)-**25** in MDCK-MDR1 cells was later evaluated to be 19. The total brain concentration was as shown ~4 μM but the fraction unbound in brain was small 0.57% indicating that these amidines display large non-specific binding to brain tissue.[19] The free brain concentration of (*R*)-**25** was determined and was judged to be sufficient to show a decrease in brain Aβ40 level if dosed together with the Pgp/BCRP inhibitor. The brain Aβ40 level was reduced by 17% in wild type mice 1.5 h after oral co-administration of Pgp/BCRP inhibitor (90 μmol/kg) and (*R*)-**25** (300 μmol/kg), as shown in Figure 5, and the plasma Aβ40 level by 76%.[18]

In summary, during the effort to optimize the aminoimidazole series towards in vivo brain efficacy we found a way to considerably improve the Caco-2 permeability properties by converting them into the di-F-tetrahydroimidazopyrimidine scaffold. In spite of reasonable Caco-2 permeability properties they still exhibited large efflux. A way to reduce efflux, as described, was to move away from the bi-aryl motif by replacing the second aryl with a linear chain. Compound (*R*)-**25** with low nM affinity for BACE-1 and low efflux in Caco-2 was examined in vivo, with co-administration of GF120918 (*R*)-**25** displayed a statistically significant Aβ40 lowering effect in mice brain.

Acknowledgments

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Supplementary data

Supplementary data (the crystallization, X-ray data collection and structure) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.079.

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8. *BACE-1 TR-FRET assay*: Soluble part of the human β-Secretase (AA 1–AA 460) and substrate (Europium)CEVNLDAEFK(Qsy7) was mixed in reaction buffer (Na-acetate, chaps, triton X-100, EDTA pH 4.5). β-Secretase were mixed with compound in DMSO and pre-incubated for 10 min. Substrate was added and the reaction allowed to proceed for 15 min at rt. The reaction was stopped with 7 μL Na-acetate, pH 9. The fluorescence of the product was measured on a Victor II plate reader with an excitation wavelength of 340 nm and an emission wavelength of 615 nm. The final concentration of the enzyme was 2.7 μg/mL; the final concentration of substrate is 100 nM. Reported values are means of $n \geq 2$ determinations, standard deviation ≤±10%.
9. Caco-2 cells grown for 14–21 days were used for the transport experiments. For both apical to basolateral (A–B) and basolateral to apical (B–A) transport directions the pH was adjusted to 7.4 with HBSS-HEPES. All compounds were investigated at a concentration of 10 μM. Buffer volumes in the 24-well plates were 0.20 mL on the apical side and 0.80 mL on the basolateral side. Samples were withdrawn after 60 min from both sides. The integrity of the epithelial

cell monolayer was monitored by measuring the passive transmembrane diffusion of [^{14}C]mannitol. Concentrations of compounds in donor and receiver samples were analyzed by liquid chromatography tandem mass spectrometry. Liquid scintillation was used for analysis of [^{14}C]mannitol. The apparent permeability coefficient Papp was calculated according to Papp = (dQ/dt)/(A * C0), where dQ/dt is the slope at 60 min of the graph of the cumulative amount transported vs time, A is the surface area of the membrane, and C0 is the starting concentration. The efflux ratio is the ratio Papp BA/Papp AB.

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12. *sAPPβ release assay*: SH-SY5Y cells were cultured in DMEM/F-12 with Glutamax, 10% FCS and 1% non-essential amino acids. Compound was incubated with cells for 16 h at 37 °C, 5% CO_2. Meso Scale Discovery (MSD) plates were used for the detection of sAPPβ release. MSD sAPPβ plates were blocked in 3% BSA in Tris wash buffer for 1 h in rt and washed four times in Tris buffer. After incubation, 20 μL of medium was transferred to the pre-blocked and washed 384 well MSD sAPPβ microplate, incubated with shaking in rt for 2 h followed by washing four times in Tris buffer. 10 μL detection antibody was added (1 nM) followed by incubation with shaking in rt for 2 h followed by washing four times in Tris buffer. 40 μL Read Buffer was added per well and the plates were read in a SECTOR Imager. In addition, 20 μL medium from the cell plates were used to analyse cytotoxicity using the ViaLight™ Plus cell proliferation/cytotoxicity kit (Cambrex BioScience) according to the manufacturer's instructions. Reported values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10$.
13. The experimental details are described in the attached Supplementary data. The coordinates and structure factors for the complexes of BACE-1 with 14 and 23, respectively, have been deposited in the Protein Data Bank (www.rcsb.org) under accession codes 4acu and 4acx.
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18. Female 9-week old C57BL/6 mice (n = 12/group) received the Pgp/BCRP inhibitor GF120918 (90 μmol/kg, po) 15 min prior to administration of vehicle or the BACE-1 inhibitor (*R*)-**25**, given at 300 μmol/kg as a single dose via oral gavage. Animals were anaesthetized 1.5 h after final administration of vehicle or (*R*)-**25**. Blood samples were collected and brains dissected for analysis of compound exposure and level of Aβ40 in plasma and brain. The blood was collected by heart puncture into pre-chilled microtainer tubes containing EDTA. Plasma samples were prepared by centrifugation for 10 min at approximately 3000 g at 4 °C within 20 min from sampling and stored frozen at −70 °C until analysis. After blood sampling, the animals were sacrificed by decapitation and brains were dissected. Cerebellum and olfactory bulbs were removed and cerebrum was divided into left and right hemispheres. The right hemisphere was weighed snap-frozen in liquid nitrogen and stored at −70 °C until exposure analysis. The left hemisphere was weighed snap-frozen in liquid nitrogen and stored at −70 °C until Aβ40 analysis. Prior to analysis, soluble Aβ was extracted according to a standardized method using diethylamine. Drug concentration in brain samples was determined by reversed-phase liquid chromatography and electro spray tandem mass spectrometry. Aβ40 levels in brain and plasma were analysed using commercial ELISA.
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