Bioorganic & Medicinal Chemistry Letters 22 (2012) 1854–1859

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

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

Britt-Marie Swahn [a,*], Jörg Holenz [a], Jacob Kihlström [a], Karin Kolmodin [a], Johan Lindström [a], Niklas Plobeck [a], Didier Rotticci [a], Fernando Sehgelmeble [a], Marie Sundström [a], Stefan von Berg [a], Johanna Fälting [b], Biljana Georgievska [b], Susanne Gustavsson [b], Jan Neelissen [c], Margareta Ek [d], Lise-Lotte Olsson [d], Stefan Berg [a,*]

[a] Department of Medicinal Chemistry, AstraZeneca R&D Södertälje, SE-15185 Södertälje, Sweden
[b] Department of Neuroscience, AstraZeneca R&D Södertälje, SE-15185 Södertälje, Sweden
[c] Department of DMPK, AstraZeneca R&D Södertälje, SE-15185 Södertälje, Sweden
[d] Discovery Sciences, AstraZeneca R&D Mölndal, SE-43183 Mölndal, Sweden

ARTICLE INFO

Article history:
Received 9 December 2011
Revised 20 January 2012
Accepted 21 January 2012
Available online 28 January 2012

Keywords:
Alzheimer's disease (AD)
β-Secretase
BACE-1 inhibitors
Aminoimidazoles
Efflux
In vivo brain efficacy

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.

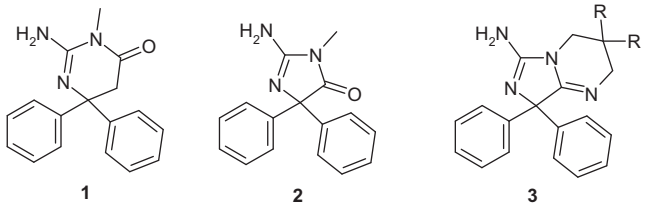
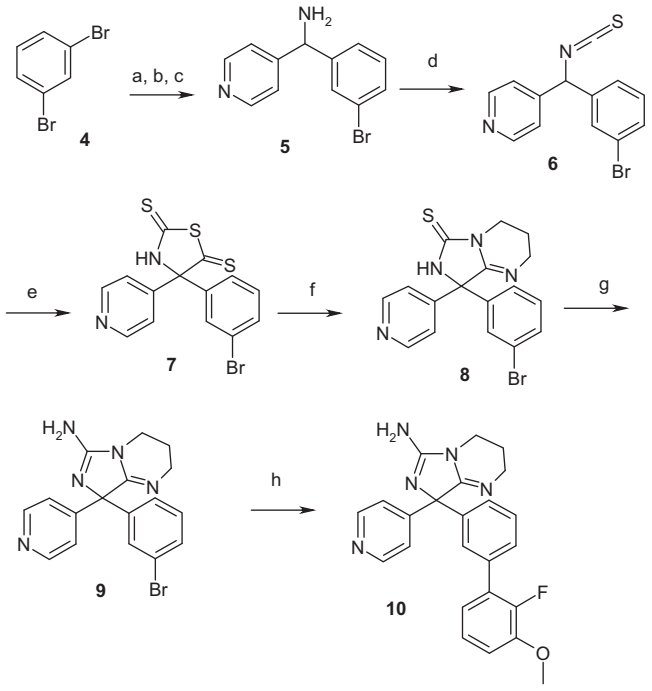


Figure 1. Scaffold hopping from dihydroisocytosines to aminohydantoins to aminoimidazoles.

* Corresponding authors.
E-mail address: britt-marie.swahn@astrazeneca.com (B.-M. Swahn).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.01.079



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.

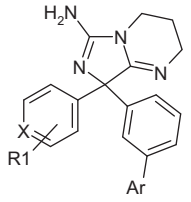
The polar surface area (PSA) of the compounds was in an acceptable range for passing the blood brain barrier (BBB), so we reasoned that the low permeability could be due to the high basicity of the aminoimidazole group (pK_a estimated to be >8) and that it is the fraction of non-protonated species that 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%.