Bioorganic & Medicinal Chemistry Letters 19 (2009) 4711–4714

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of BACE-1 inhibitors utilizing a tertiary hydroxyl motif as the transition state mimic

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ARTICLE INFO

Article history:
Received 6 May 2009
Revised 16 June 2009
Accepted 16 June 2009
Available online 21 June 2009

Keywords:
Alzheimer's disease
BACE-1
Enzyme inhibitor
Transition state mimetic

ABSTRACT

Two series of drug-like BACE-1 inhibitors with a shielded tertiary hydroxyl as transition state isostere have been synthesized. The most potent inhibitor exhibited a BACE-1 IC_{50} value of 0.23 μM.

Alzheimer's disease (AD) is the most common form of dementia among the elderly and it is currently believed that approximately 37 million people worldwide have the disease.[1] Two hallmarks for AD are accumulation and extracellular aggregation of insoluble amyloid plaques (Aβ) deposits, along with intracellular neurofibrillary tangles in the brain. The human aspartic protease BACE-1 has long been regarded as a therapeutic target for the reduction of Aβ formation.[2] BACE-1 is the initial protease that processes amyloid precursor protein (APP) in the pathway leading to Aβ.[3] The finding that BACE-1 knockout mice were unable to produce Aβ provided an in vivo validation of BACE-1 as one of the proteases responsible for Aβ production in the brain.[4,5] Thus, inhibition of BACE-1 is a promising strategy in the search for an effective AD treatment.

Inhibition of aspartic proteases has been challenging and in the case of BACE-1 the first generation of inhibitors were inspired by knowledge gained in the development of HIV-1 protease and renin inhibitors.[6] The first publically available enzyme/inhibitor co-crystal X-ray structure (PDB-code: 1FKN) further spurred the discovery process.[7] Despite extensive research and even though numerous potent and selective inhibitors have been found, no BACE-1 inhibitors have yet reached the market. Several reported inhibitors suffer from poor bioavailability, insufficient membrane permeability and blood-brain barrier penetration due to their peptidic character.[1,4]

Previous work from our laboratory has demonstrated that HIV-1 protease inhibitors containing a shielded tertiary hydroxyl group as transition state mimic have improved Caco-2 cell membrane permeability while retaining good inhibitory properties.[8] Inspired by these results, we sought to develop BACE-1 inhibitors containing a tertiary hydroxyl group as the central transition state isostere, together with a benzyl or a phenylethylene group in the P1-position. A flexible and robust synthetic route was developed, which gave access to two different tertiary statine-like moieties (Fig. 1). Both structural classes were used in combination with a substituted isophthalamide containing an inverted amide bond and various selected substituents in the P2′-P3′ position, designed to bind into the enzyme subsites S2′-S3′.

The synthesis of the final inhibitors is depicted in Scheme 1.[9] Starting by protection of 2-hydroxy-3-phenyl-propionic acid ($n = 1$) or 2-hydroxy-4-phenyl-butyric acid ($n = 2$) to render ketals

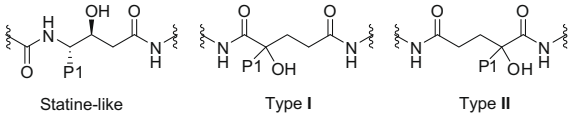

Figure 1. A comparison between transition state isosteres, the commonly used statine motif and the two transition state cores discussed in this report. Both type **I** and **II** uses a tertiary hydroxyl group in the transition state mimic.

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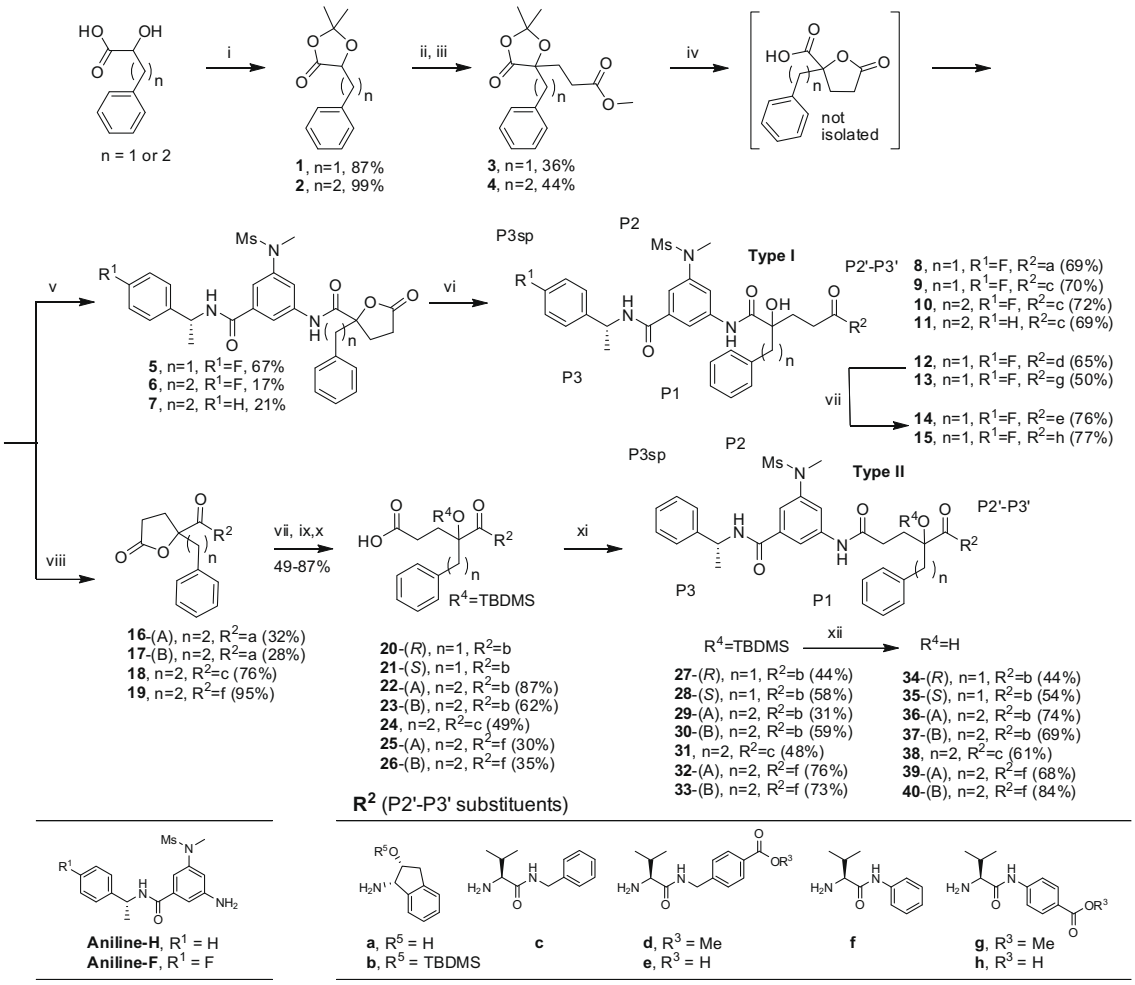
doi:10.1016/j.bmcl.2009.06.065

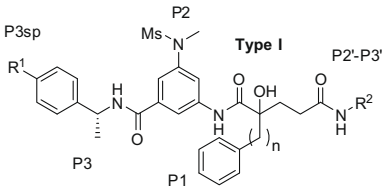


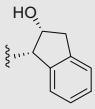
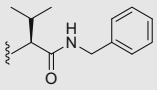
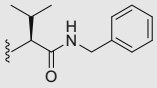
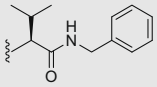
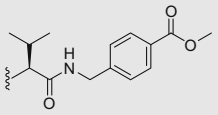
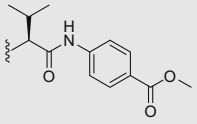
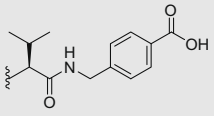
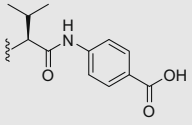
Scheme 1. Reagents and conditions: (i) Pyridinium *p*-toluenesulphonic acid, dimethoxy propane, $CHCl_3$, 70 °C; (ii) LDA, THF (dry), −78 °C; (iii) methyl acrylate; (iv) TFA/H_2O (6:1), 80 °C; (v) **aniline-H** or **aniline-F**, EDC, HOBt, DIPEA, CH_2Cl_2 (dry), 40 °C; (vi) R^2, 2-hydroxypyridine, DIPEA, THF (dry), reflux; (vii) LiOH, dioxane/H_2O (1:1), rt; (viii) R^2, EDC, HOBt, DIPEA, CH_2Cl_2 (dry), 40 °C; (ix) TBDMS-OTf, Et_3N, DCM/THF (1:1), rt; (x) 1 M K_2CO_3 (aq), THF, MeOH (1:1:3), rt; (xi) **aniline-H**, EDC, HOBt, DIPEA, CH_2Cl_2 (dry); (xii) TBAF, THF, rt.

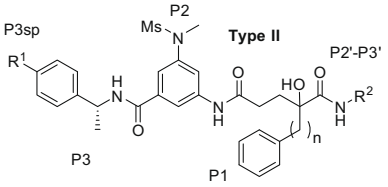
1 (87%) and **2** (99%), respectively. The quaternary center was introduced by alkylation of compounds **1** and **2** with methyl acrylate in a Michael addition to give the intermediates **3** (36%) and **4** (44%), essentially as previously described procedure.[10] For the synthesis of type **I** final products, intermolecular lactone formation was performed by heating the esters **3** and **4** in aqueous trifluoracetic acid. The acid was then coupled with **aniline-H** (R^1 = H)[11] or **aniline-F** (R^1 = F)[9] using EDC, HOBt and DIPEA in dry CH_2Cl_2 to give compounds **5**–**7** (17–67%). Lactones **5**–**7** was further ring opened with a selected set of amines (**a**, **c**, **d**, **g**) in the presence of DIPEA and 2-hydroxypyridine in refluxing THF, to give the final type **I** inhibitors in 50–72% yield (**8**–**13**, Scheme 1 and Table 1). Amine **a** is commercially available while amines **c**, **d**, and **g** were synthesized according to standard procedures.[9] The methyl ester in products **12** and **13** was hydrolyzed to furnish inhibitors **14** and **15**, containing a free carboxylic acid in 76–77% yield. The inhibitors **8**–**15** were isolated and evaluated for BACE-1 inhibition as diastereomeric mixtures (1:1). To synthesize inhibitors of type **II**, the acids generated from the intermolecular lactonization of **3** and **4** were subjected to standard peptide coupling chemistry (EDC, HOBt, and DIPEA in dry CH_2Cl_2) with three different amines **a**, **c**, and **f** to furnish lactones **16**–**19** in 60–95% yield. When (1*S*,2*R*)-1-aminoindanol (**a**) was used, the formed diastereomers could be separated by flash column chromatography to yield the pure diastereomers **16**-(A) and **17**-(B) in 32% and 28% yield, respectively. The lactones **16**–**19** were opened with LiOH and the generated tertiary alcohols were protected with TBDMS-OTf to provide the corresponding acids **22**–**26** in 30–87% yield over three steps. To obtain compounds **25**-(A) and **26**-(B) as pure diastereomers, the corresponding diastereomeric acid was purified by preparative HPLC. Structures **20**-(*R*) and **21**-(*S*) were prepared according to a literature procedure.[10] Next, acids **20**–**26** were reacted with **aniline-H** using standard peptide coupling conditions to afford the TBDMS-protected compounds **27**–**33** in 44–76% yield. Finally, to complete the synthesis of type **II** inhibitors the TBDMS-protecting group was removed using TBAF in THF to furnish the final inhibitors **34**–**40** in 44–84% yield (Table 1).

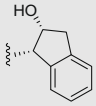
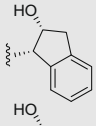
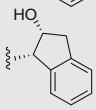
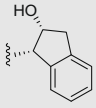
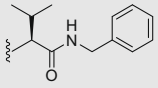
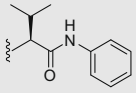
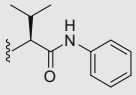
All synthesized inhibitors were screened against BACE-1 to determine IC_{50} values (Table 1).[12] The optimized and previously used isophthalamide moiety[13] was exclusively used in the synthesized inhibitors with two modifications, an inverted amide bond next to the novel tertiary hydroxyl transition state mimic and in some examples with fluorine in the P3sp *para* position instead of hydrogen. This P2-P3 group together with the indanolamine (**a**) as P2′-P3′ substituent, previously used in the development of HIV-1 protease inhibitors, gave an inhibitor with no BACE-1 activity (**8**, IC_{50} >10 μM). Replacing the indanolamine with the previously used P2'-P3' BACE-1 substituent, Val-benzylamide[4] (**c**), furnished an inhibitor with modest BACE-1 inhibitory data (**9**, IC_{50} = 5.9 μM). The weak inhibitory properties were lost when an

Table 1
BACE-1 inhibition data



Compd	*n*	R^1	R^2	BACE-1 IC_{50} (μM)
8	1	F		>10
9	1	F		5.9
10	2	F		>10
11	2	H		5.6
12	1	F		>10
13	1	F		4.0
14	1	F		>10
15	1	F		>10



Compd	*n*	R^1	R^2	BACE-1 IC_{50} (μM)
34-(*R*)	1	H		4.6
35-(*S*)	1	H		2.5
36-(A)	2	H		0.57[a]
37-(B)	2	H		0.25[a,b]
38	2	H		0.45[a]
39-(A)	2	H		0.23[a,c,d]
40-(B)	2	H		0.32[a,d]

[a] CatD K_i >5000 nM.
[b] P_{app} (Caco-2) <1 × 10^{-6} cm/s.
[c] P_{app} (Caco-2) = 1.2 × 10^{-6} cm/s.
[d] CatD was determined on a diastereomeric mixture (1:1).

elongation by one carbon was incorporated in the P1 side-chain to provide **10** (>10 μM). Changing fluorine to hydrogen in the *para* P3sp position restored the inhibitory properties, cf. **10** and **11** (>10 μM and 5.6 μM). Several of the previously reported statine containing BACE-1 inhibitors need an acidic functionality in the P3′ position for potent BACE-1 inhibition.[12] Introduction of an acidic functionality in our inhibitors did not furnish inhibitors with improved activity, **14** (>10 μM) and **15** (>10 μM). The corresponding ester analogues were in fact found to be slightly better; compare **14** and **15** with the methyl esters **12** and **13** (BACE-1 IC_{50} >10 μM and 4.0 μM). All the inhibitors described above contain the type **I** tertiary transition state mimic, representing a structural change to a tertiary hydroxyl group at the same carbon as the presumed P1 substituent (Fig. 1). Evidently, this modification was not well tolerated in the active site of the BACE-1 protease. However, the central core contains two available protected carboxylic acid functionalities which were used orthogonally to give inhibitors of the generic type **II** (Scheme 1). This modification rendered compounds with an improved activity against BACE-1 (Table 1). Introducing the indanolamine (**a**) in the P2-P3 position together with the benzyl moiety in the P1-position furnished compounds **34**-(*R*) and **35**-(*S*) (IC_{50} = 4.6 μM and 2.5 μM). The absolute stereochemistry of **34**-(*R*) and **35**-(*S*) was determined by X-ray crystallography data (PDB-code: 2UY0) from HIV-1 protease inhibitors synthesized from the intermediates **20**-(*R*) and **21**-(*S*).[10] A one carbon elongation in the P1-position improved the activity of this scaffold, compare **36**-(A) (IC_{50} = 0.57 μM) and **37**-(B) (IC_{50} = 0.25 μM) versus **34**-(*R*) and **35**-(*S*). Attaching the common Val-benzylamide (**c**) in the P2′-P3′ position provided an equipotent inhibitor, **38** (BACE-1 IC_{50} = 0.45 μM) while replacing the benzylamine with an aniline in the P3′ position, Val-phenylamide (**f**), afforded the pure diastereomeric inhibitors **39**-(A) and **40**-(B) with BACE-1 IC_{50} values of 0.23 μM and 0.32 μM, respectively. The epimers **39**-(A) and **40**-(B) were equally potent which suggests that in this

series of shielded BACE-1 inhibitors the absolute stereochemistry at the quaternary center is of minor importance. The most potent inhibitors from this series were also screened for inhibition of the anti-target human cathepsin D (Table 1) and were found to be selective towards BACE-1 (CatD K_i >5000 nM).[12] Cell membrane permeability analysis (Caco-2) of **37**-(B) and **39**-(A) revealed that this class of inhibitors have relatively low membrane permeability ($<1 \times 10^{-6}$ and 1.2×10^{-6} cm/s, respectively).

The described synthetic route provided two types of masked tertiary hydroxyl central cores which were smoothly diversified with different substituents. In total 15 BACE-1 inhibitors were synthesized. The most potent inhibitor **39**-(A) exhibited a BACE-1 value of 0.23 μM and was selective towards CatD, but showed only low cell permeability. This result reveals that this class of inhibitors in the future needs to be further optimized in order to produce inhibitors in the nanomolar range with good pharmacokinetic properties.

Acknowledgments

Dr. Xiongyu Wu for providing compounds **20**-(*R*) and **21**-(*S*). Dr. Elizabeth Hamerlink and Dr. Ian Henderson for BACE-1 and CatD enzyme data, Alexandra Johansson, and Elisabet Lilja for excellent technical assistance in the production of BACE-1, Dr. Anders Blomqvist for the original cloning of the human gene for BACE-1. Aleh Yahorau for conducting the HRMS experiments. Dr. Luke Odell for linguistic improvements. Finally, we would like to acknowledge the Swedish Research Council, Alice and Knut Wallenberg's Foundation and Medivir AB for Caco-2 permeability analyses and financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.065.

References and notes

1. Mount, C.; Downton, C. *Nat. Med.* **2006**, *12*, 780.
2. Cole, S. L.; Vassar, R. *Curr. Alzheimer Res.* **2008**, *5*, 100.
3. Ghosh, A. K.; Gemma, S.; Tang, J. *Neurotherapeutics* **2008**, *5*, 399.
4. Silvestri, R. *Med. Res. Rev.* **2009**, *29*, 295.
5. Sisodia, S. S.; St George-Hyslop, P. H. *Nat. Rev. Neurosci.* **2002**, *3*, 281.
6. Eder, J.; Hommel, U.; Cumin, F.; Martoglio, B.; Gerhartz, B. *Curr. Pharm. Des.* **2007**, *13*, 271.
7. Hong, L.; Koelsch, G.; Lin, X. L.; Wu, S. L.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. *Science* **2000**, *290*, 150.
8. Ekegren, J. K.; Unge, T.; Safa, M. Z.; Wallberg, H.; Samuelsson, B.; Hallberg, A. *J. Med. Chem.* **2005**, *48*, 8098.
9. Experimental details and spectroscopic data can be found in the Supplementary data associated with this article, available online at sciencedirect.com.
10. Wu, X. Y.; Öhrngren, P.; Ekegren, J. K.; Unge, J.; Unge, T.; Wallberg, H.; Samuelsson, B.; Hallberg, A.; Larhed, M. *J. Med. Chem.* **2008**, *51*, 1053.
11. Russo, F.; Wångsell, F.; Sävmarker, J.; Jacobsson, M.; Larhed, M., submitted for publication.
12. Bäck, M.; Nyhlen, J.; Kvarnström, I.; Appelgren, S.; Borkakoti, N.; Jansson, K.; Lindberg, J.; Nyström, S.; Hallberg, A.; Rosenquist, S.; Samuelsson, B. *Bioorg. Med. Chem.* **2008**, *16*, 9471.
13. Stachel, S. J.; Coburn, C. A.; Steele, T. G.; Jones, K. G.; Loutzenhiser, E. F.; Gregro, A. R.; Rajapakse, H. A.; Lai, M. T.; Crouthamel, M. C.; Xu, M.; Tugusheva, K.; Lineberger, J. E.; Pietrak, B. L.; Espeseth, A. S.; Shi, X. P.; Chen-Dodson, E.; Holloway, M. K.; Munshi, S.; Simon, A. J.; Kuo, L.; Vacca, J. P. *J. Med. Chem.* **2004**, *47*, 6447.