Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 48 (2007) 7990–7993
Tetrahedron Letters

Aspartic protease inhibitors via C_1-homologation of peptidic aldehydes and studies on reduced amide isosteres

Hannes A. Braun,[a] Andrea Zall,[a] Manfred Brockhaus,[b] Marco Schütz,[a] Reinhard Meusinger[a] and Boris Schmidt[a,*]

[a]*Clemens Schöpf Institute for Organic Chemistry and Biochemistry, Darmstadt University of Technology, Petersenstr. 22, 64287 Darmstadt, Germany*
[b]*Department of PRBD-N Neuroscience, F. Hoffmann-La Roche, 4070 Basel, Switzerland*

Received 29 June 2007; revised 29 August 2007; accepted 7 September 2007
Available online 12 September 2007

Abstract—(*R*)-Configured isophthalic hydroxyethylamines play an important role in the inhibition of β-secretase (BACE1). We present the synthesis of a number of (*S*)-configured hydroxyethylamine derivatives via 2-iodoethanol intermediates and the comparison with the (*R*)-analogues. An *N*-substituted indole was investigated as a substitute for the isophthalamide moiety.

Extracellular amyloid-β plaques are suspected to cause Alzheimer's disease.[1,2] These plaques consist mainly of amyloid-β peptides (Aβ) which are generated from the membrane bound APP (amyloid precursor protein) via the consecutive cleavages by β-secretase (BACE1, β-site amyloid precursor protein cleaving enzyme) and γ-secretase.[3] Thus, the inhibition of BACE1 constitutes a promising approach to block the onset of plaque formation by decreasing the Aβ levels in the brain.

A number of highly potent, but peptidic BACE1 inhibitors have been available for several years.[4,5] Inhibitors **1**–**3** have derived from the progressive elimination of peptidic features from the original heptapeptidic hydroxyethylenes (Fig. 1). The introduction of an isophthalic moiety in **1** was regarded as a milestone in the development of small BACE1 inhibitors.[6] The peptidic character of compound **1** was further reduced by the replacement of the hydroxyethylene with a hydroxyethylamine. Remarkably, the hydroxyethylamine in **2** is the only transition state isostere in BACE1 inhibitors that displays an (*R*)-configured secondary alcohol.[7] Compound **3** comprises a reduced amide transition state isostere with an additional sulfonamide

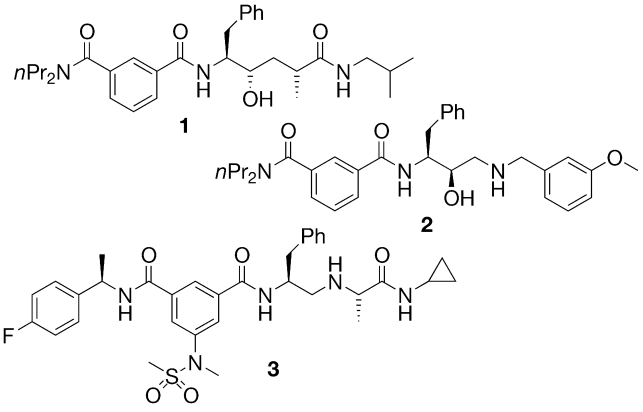

Figure 1. BACE1 inhibitors by Elan pharmaceuticals and MSD.

substituent in the P2 position and a modified P3 amide residue.[8] Compounds **1**–**3** are active at nanomolar concentrations in cell free assays and yet display different cellular activities. They are good substrates for the p-glycoprotein transporter, this was partially assigned to the isophthalic amide moiety.[9]

Here, we present an approach to isophthalamide based hydroxyethylamines and potential replacements thereof. We compared a linear to a convergent access to this class of compounds. The synthesis led to the (*S*)-configured secondary alcohols which were compared to the known (*R*)-analogue **2**. Furthermore, we investigated

Keywords: β-Secretase; Transition state isostere; Hydroxyethylamine; Reduced amide; Grignard reagent; Indole synthesis.
* Corresponding author. Tel.: +49 6151 163075; fax: +49 6151 163278; e-mail: schmidt_boris@t-online.de

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.09.047

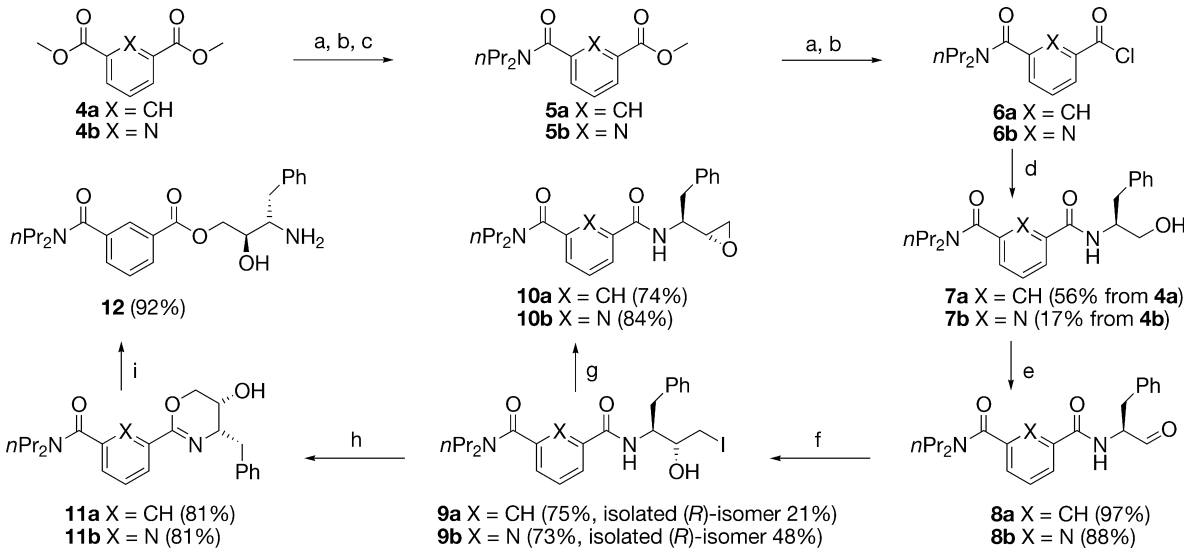

Figure 2. Synthesis of epoxides **10a** and **10b** and ester **12**. Reagents and conditions: (a) KOH, MeOH, rt; (b) $SOCl_2$, $CHCl_3$, reflux, (overnight); (c) *n*-Pr_2NH, CH_2Cl_2, 0 °C to rt; (d) L-phenylalaninol, CH_2Cl_2, 0 °C to rt; (e) IBX, DMSO, rt, 15 h; (f) *i*-PrMgCl, CH_2I_2, THF, −78 °C (15 min), 0 °C (2 h); (g) K_2CO_3, MeCN, rt (4 h); (h) 4-bromobenzylamine, MeCN, reflux (4 h); (i) water, THF, trifluoroacetic acid, 80 °C (1 h), rt (15 h).

an indole as a replacement for the isophthalamide portion, combined it with a reduced amide isostere and compared it to the isophthalamide analogue.

A reaction sequence starting from diester **4a** was employed for the generation of **5a**, **6a** and finally the isophthalamide **7a** comprising two different amide substituents on either carboxyl functionality (Fig. 2).[10] Alcohol **7a** was oxidized by IBX (2-iodooxybenzoic acid) furnishing aldehyde **8a** in a high yield and free of racemisation. The aldehyde was converted to a 2-iodoethanol using a moderately stereoselective C_1-homologation.[11] The reaction occurs upon treatment with *i*-PrMgCl/CH_2I_2 forming a mild Grignard reagent that undergoes addition to peptidic aldehydes in a high chemoselectivity leaving the α-chiral position unchanged. Product **9a** (crude ds = 3:1) was obtained in 75% yield as a diastereomeric mixture and further purification delivered pure **9a**. Treatment of **9a** with K_2CO_3 yielded epoxide **10a**. Surprisingly, **9a** was converted to oxazine **11a** upon treatment with any benzylamine in MeCN under reflux. **11a** could be opened to ester **12** by treatment with trifluoroacetic acid in a water/THF mixture. A small amount of the diastereomer of **11a** was generated likewise, and the cyclic structures of both diastereomers of **11a** served to assign the configuration of compounds **9**–**12**. The reactions to the analogous compounds **5b**–**11b** starting from dimethyl 2,6-pyridinedicarboxylate **4b** were conducted in a similar manner. The higher yield of **9b** (de = 100%) compared to **9a** is explained by the higher diastereoselectivity of the C_1-homologation (crude ds = 9:1). Even though the generation of **9a** or **9b** seemed to be a promising approach for a variety of benzylic hydroxyethylamines, the iodo compounds reacted under a variety of conditions to the undesired oxazines. This undesired reaction was circumvented in previous synthetic approaches:[12] (a) by the protection of the amide nitrogen or (b) by the conversion of an *N*-Boc-protected amino acid derivative (e.g., **15**) or its respective epoxide, which does not undergo cyclization. This difference in carbamate versus amide reactivity was applied to the synthesis of the desired hydroxyethylamines. Boc-phenylalaninol **13** was oxidized to **14** with IBX and converted to β-iodoethanols **15a** and **15b** (de >99%, crude ds = 3:2, Fig. 3).

Either diastereomer was obtained in high purity from the crude diastereomeric mixture (53%). Substitution of the iodide by various benzylamines provided hydroxyethylamines **16**. Deprotection and reaction with chloride **6a** led to the desired (*S*)-configured alcohols **17**–**20** and (*R*)-alcohol **2**. The configuration was assigned by single crystal X-ray analysis of **15a** and subsequent conversions of **15b** to known compounds and comparison of the NMR data.[11]

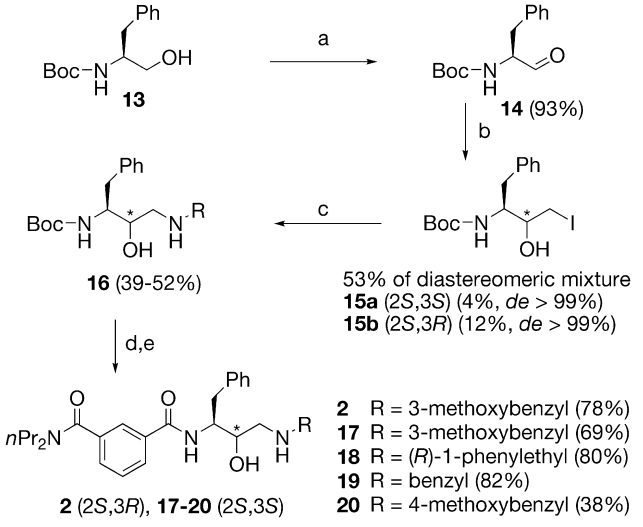

Figure 3. Reagents and conditions: (a) IBX, DMSO, rt (15 h); (b) *i*-PrMgCl, CH_2I_2, THF, −78 °C (15 min), 0 °C (2 h); (c) amine, MeCN, reflux (4 h); (d) trifluoroacetic acid, CH_2Cl_2; (e) **6a**, HOBt, CH_2Cl_2 (overnight).

Epoxides **10a**, **10b**, oxazine **11a**, ester **12** and hydroxyethylamines **2**, **17**–**20** were tested in BACE1-assays (fluorescence resonance energy transfer (FRET) assay,[13] RLBA (radioactive ligand binding assay)[14] in the presence of detergent (Tween 20) or serum albumin (BSA)) and a FRET Cathepsin D assay[13] (Table 1). The epoxides proved to be inhibitors equally of both BACE1 and Cathepsin D. In further studies using the RLBA assay compound **10a** showed an irreversible, time-dependent inhibition. The apparent IC_{50} was 39 μM after 10 min and 3 μM after 60 min incubation. The same compound was not active in a cellular system measuring Aβ production by HEK293 cells. Compound **11a** was investigated because of the structural similarity to an oxazole reported by Rajapakse et al.[15] While the oxazole was inactive, its hydrolyzed linear form was a highly potent inhibitor. Similar to the oxazole, oxazine **11a** displayed no inhibition of BACE1 and its ring-opened derivative **12** was a moderate inhibitor of BACE1. Interestingly, the (*S*)-configured hydroxyethylamines displayed no inhibition of BACE1. This proves the importance of the opposite (*R*)-configuration on the secondary alcohol in hydroxyethylamines compared to other transition state isosteres.

In search of an isophthalamide replacement we and others[12,16] hypothesized that the indole nitrogen in a (4-amino-6-carboxyl)-indole (e.g., **23**) could be attached to an alkyl substituent mimicking the P3 residue, while the amine functionality can be converted into a sulfonamide and the carboxylate serves as a linker to a transition state isostere. Such indoles display H-bonds and thus may exhibit a reduced affinity for the p-glycoprotein transporter[9] and the enhanced rigidity might increase their activity. The synthesis started from the C_2-symmetrical acid **21** (Fig. 4). After esterification, an enamine was generated that underwent cyclization to the indole in a subsequent Pd/C catalyzed reduction.[17] The resulting amine was converted into a methyl sulfonamide and the amide nitrogen was methylated after selective deprotonation. After a second deprotonation, the indole nitrogen was alkylated with *i*-BuI and the ester was saponified to furnish building block **25**.

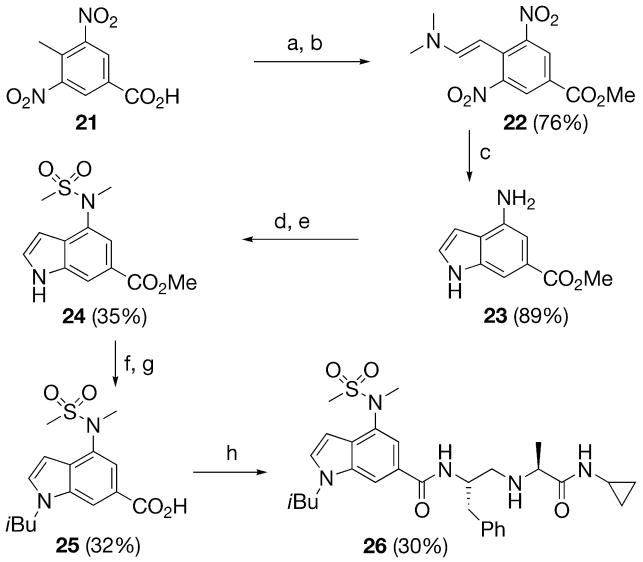

Figure 4. Reagents and conditions: (a) (i) $SOCl_2$, $CHCl_3$, reflux (overnight) (ii) MeOH, Et_3N, CH_2Cl_2, 0 °C to rt; (b) $HC(NMe_2)(OMe)_2$, CuI, DMF, microwave (180 °C, 10 min); (c) Pd/C, H_2, MeOH, 3 d; (d) $MeSO_2Cl$, THF, rt (4 h); (e) NaH, MeI, THF, rt (1 h); (f) K_2CO_3, *i*-BuI, DMF, 80 °C (2 d); (g) KOH, MeOH, reflux (2 d); (h) (i) EDAC, HOBt, CH_2Cl_2, (ii) **29b**, Et_3N.

A reduced amide transition state isostere was chosen for elongation. Coburn et al.[8] reported on a variety of reduced amides similar to **3** and two of the most active isosteres were generated via reductive amination based on a Z-strategy. Z-L-alanine **27** was coupled with *i*-$BuNH_2$ or cyclopropylamine (c-$PrNH_2$) to the respective amides (Fig. 5). The resulting amides were deprotected and converted to **28a** and **28b** by treatment with *N*-Z-L-phenylalaninal and $Na(AcO)_3BH$. The primary amines were deprotected to provide the transition state isosteres **29a** and **29b**. Compound **29b** was coupled to indole **25** in a final step leading to inhibitor **26**. The secondary amine **29a** was coupled with **6a** to provide compound **30** in order to compare the activity of indole **26** with other P2–P3 mimetics. Indole **26** is active at micromolar concentrations and exhibits a similar activ-

Table 1. IC_{50} of compounds **2**, **10a**, **10b**, **11a**, **12**, **17**–**20**, **26** and **30**

Compounds	BACE1			CatD
	FRET IC_{50} (μM)	RLBA (tween) IC_{50} (μM)	RLBA (BSA) IC_{50} (μM)	FRET IC_{50} (μM)
2	1.2	0.14	0.19	0.1
10a	106	10.4	11.0	63.0
10b	95.7	15.4	16.2	194.2
11a	—	—	—	197.6
12	91.1	37.9	36.4	39.0
17	76.5	34.2	37.9	19.3
18	>200	—	—	63.3
19	200	200	127	12.3
20	—	—	—	69.2
26	35	25	20	75
30	17.0	4.6	8.6	84.9

No inhibition: —.

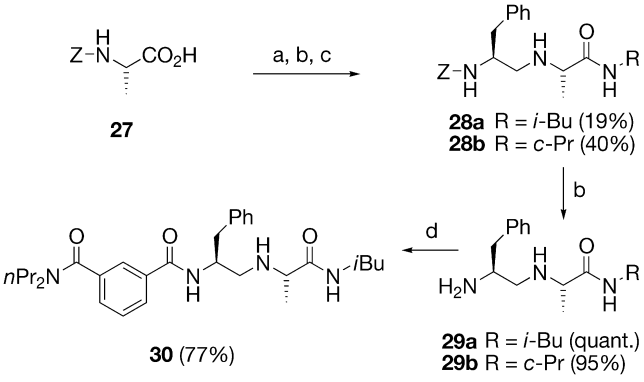

Figure 5. Reagents and conditions: (a) (i) EDAC, HOBt, CH_2Cl_2, (ii) amine, Et_3N; (b) Pd/C, H_2, MeOH, 3 d; (c) *N*-Z-L-phenylalaninal, $Na(AcO)_3BH$, Et_3N, $MgSO_4$, CH_2Cl_2, rt (overnight); (d) **6a**, HOBt, CH_2Cl_2, rt (15 h).

ity like isophthalamide **30** (Table 1). Surprisingly, this results in an approximately 1000-fold decreased activity compared to amide **3** ($IC_{50} = 24$ nM).

In conclusion, we confirmed the (*S*)-configured hydroxyethylamines to be inactive and thus to constitute an exception to all other transition state isosteres employed in BACE1 inhibition. However, (*S*)-configured hydroxyethylamines are known as isosteres in inhibitors targeting other aspartyl proteases. The depicted C_1-homologation resulted in useful intermediates for such protease inhibitors. The direct nucleophilic opening of acyl N-protected α-aminoepoxides is an attractive synthesis to bioactive molecules, but turned out to be inaccessible. However, the initially undesired cyclization allowed us to unambiguously establish the stereochemistry due to cyclic constraints. The combination of an indole bearing an *i*-butyl P3 residue and a methyl sulfonamide P2 residue proved equally potent as the *N*,*N*-dipropylisophthalamide analogue, but neither of the compounds displayed activities comparable to the most potent BACE1 inhibitors.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SPP1085 SCHM1012-3) and the EU Contract LSHM-CT-2003-503330 (APOPIS).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.09.047.

References and notes

1. Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741–766.
2. Adalbert, R.; Gilley, J.; Coleman, M. P. *Trends Mol. Med.* **2007**, *13*, 135–142.
3. Weidemann, A.; Eggert, S.; Reinhard Friedrich, B. M.; Vogel, M.; Paliga, K.; Baier, G.; Masters Colin, L.; Beyreuther, K.; Evin, G. *Biochemistry* **2002**, *41*, 2825–2835.
4. Guo, T.; Hobbs, D. W. *Curr. Med. Chem.* **2006**, *13*, 1811–1829.
5. Schmidt, B.; Baumann, S.; Narlawar, R.; Braun, H. A.; Larbig, G. *Neurodegenerative Dis.* **2006**, *3*, 290–297.
6. Hom Roy, K.; Gailunas Andrea, F.; Mamo, S.; Fang Larry, Y.; Tung Jay, S.; Walker Donald, E.; Davis, D.; Thorsett Eugene, D.; Jewett Nancy, E.; Moon Joseph, B.; John, V. *J. Med. Chem.* **2004**, *47*, 158–164.
7. Maillaird, M.; Hom, C.; Gailunas, A.; Jagodzinska, B.; Fang, L. Y.; John, V.; Freskos, J. N.; Pulley, S. R.; Beck, J. P.; Tenbrink, R. E., WO2002002512, **2002**.
8. Coburn, C. A.; Stachel, S. J.; Jones, K. G.; Steele, T. G.; Rush, D. M.; DiMuzio, J.; Pietrak, B. L.; Lai, M. T.; Huang, Q.; Lineberger, J.; Jin, L.; Munshi, S.; Katharine Holloway, M.; Espeseth, A.; Simon, A.; Hazuda, D.; Graham, S. L.; Vacca, J. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3635–3638.
9. Schinkel, A. H. *Adv. Drug Delivery Rev.* **1999**, *36*, 179–194.
10. Umbreen, S.; Brockhaus, M.; Ehrenberg, H.; Schmidt, B. *Eur. J. Org. Chem.* **2006**, 4585–4595.
11. Braun, H. A.; Meusinger, R.; Schmidt, B. *Tetrahedron Lett.* **2005**, *46*, 2551–2554.
12. Kleinman, E. F.; Murray, J. C., WO2006032999, **2006**.
13. Gruninger-Leitch, F.; Schlatter, D.; Kung, E.; Nelbock, P.; Dobeli, H. *J. Biol. Chem.* **2002**, *277*, 4687–4693.
14. Brockhaus, M.; Doebeli, H.; Grueninger, F.; Huguenin, P.; Kitas, E. A.; Nelboeck-Hochstetter, P., US2003125257, **2003**.
15. Rajapakse, H. A.; Nantermet, P. G.; Selnick, H. G.; Munshi, S.; McGaughey, G. B.; Lindsley, S. R.; Young, M. B.; Lai, M. T.; Espeseth, A. S.; Shi, X. P.; Colussi, D.; Pietrak, B.; Crouthamel, M. C.; Tugusheva, K.; Huang, Q.; Xu, M.; Simon, A. J.; Kuo, L.; Hazuda, D. J.; Graham, S.; Vacca, J. P. *J. Med. Chem.* **2006**, *49*, 7270–7273.
16. Redshaw, S.; Demont, E. H.; Walter, D. S., WO2005058915, **2005**.
17. Siu, J.; Baxendale, I. R.; Ley, S. V. *Org. Biomol. Chem.* **2004**, *2*, 160–167.