Biphenyl-Derivatives Possessing Tertiary Amino Groups as β-Secretase (BACE1) Inhibitors

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Abstract: β-Secretase (BACE1, β-site APP cleaving enzyme) is one of the most challenging therapeutic targets in the field of Alzheimer's disease (AD) research. This enzyme catalyses the formation of neuronal amyloid β (Aβ) plaques, whose increased production is a key event in the initial pathogenesis of AD. As a consequence, many BACE1 inhibitors have been developed by several research groups. In the present work, after an analysis of tetraline derivatives reported in a Takeda patent, we designed and synthesized some analogues, making appropriate structural modifications, in order to try to improve the bioavailability features and the activities of Takeda compounds. All the new derivatives were tested on BACE1 with the TR-FRET (Time Resolved-Fluorescence Resonance Energy Transfer) technology and one of them showed a promising inhibitory activity value.

Keywords: Alzheimer's disease, BACE1, Bicyclic core, Biphenyl substituent, Tetraline derivative, TR-FRET technology.

INTRODUCTION

Alzheimer's disease is an age-related disorder that is responsible for a slow and progressive neurodegeneration, especially in the hippocampal and neocortical regions. From a neuropathological point of view, this disease is characterized by the formation of extracellular senile plaques, mainly composed of amyloid β peptides (Aβ) aggregates [1], and intracellular neurofibrillary tangles, made of hyperphosphorylated tau proteins [2]. Both of these brain lesions may have a possible key role in the progression of AD and have been recognized as diagnostic hallmarks of this disease [3].

Aβ peptides are originated from a larger transmembrane protein called amyloid precursor protein (APP), that is subjected to the action of specific enzymes, such as β-secretase and γ-secretase. In particular, β-secretase (BACE1), a proteolytic enzyme belonging to the family of aspartyl proteases, catalyzes the first and rate-limiting step of the sequential cleavage of APP. Besides, it is well established that BACE1 -/- mice show no adverse phenotype and they have no detectable levels of Aβ peptides, indicating that BACE1 inhibition poses no serious consequences for the health of the mice [4]. As a result, BACE1 is considered one of the preferred therapeutic targets for the treatment of AD and there is a great interest in developing BACE1 inhibitors [5]. However, some of the more potent BACE1 inhibitors so far discovered are peptidomimetic or possess relatively high molecular weights, in spite of the fact that BACE1 inhibitors have to be able to cross blood-brain barrier (BBB) in order to reach their target in the CNS. Reduction of molecular size and generally of the peptidic nature and overall polarity of the candidate inhibitors are strategies usually applied for this purpose.

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The research of new non-peptidomimetic BACE1 inhibitors that possess appropriate properties for drug development, such as low molecular weight, good potency and high selectivity, has been started from a detailed study of a Takeda patent, in which tetraline derivatives as BACE inhibitors are described (Fig. **1**) [6].

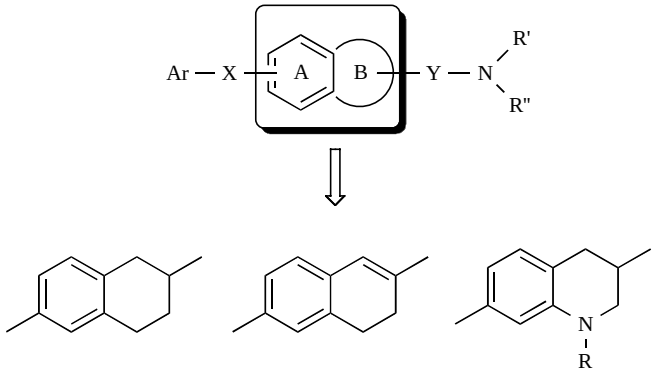

Fig. (1). General structures of compounds reported in the Takeda patent [6].

In particular, the aminoethyl-substituted tetraline derivative **1** (Fig. **2**) has been used as starting point for further structural modifications as it was described as a potent BACE1 inhibitor. However, its development as a drug may present some difficulties because of its high lipophilicity, that can compromise oral bioavailability, having a CLogP value of 8.3 [7].

On the basis of these data, we envisaged the possibility of developing a new series of BACE1 inhibitor compounds related to these tetralinic structures, but with reduced lipophilicity. The strategy was based on the replacement of the central tetralinic core, reported in the patent, with less hydrophobic moieties or heterocycles, in order to enhance the polarity of the resulting compounds. In the design of new compounds, we have kept the extremities of Takeda struc-

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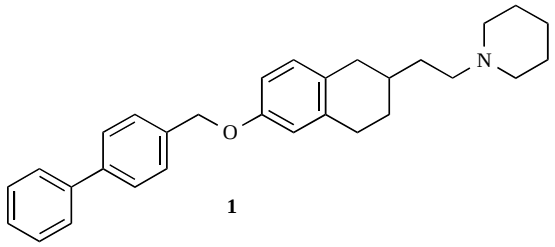


Fig. (2). Reference Takeda BACE1 inhibitor **1**.

tures constant, that are, the biphenyl scaffold and the lateral amino side chain present in the best compound (**1**, Fig. **2**). The selection of the central core replacements has been done considering synthetic accessibility of the proposed structures, leading to compounds **2-5** (Fig. **3**) as the best candidates for a further exploration. In particular, the introduction of oxygen atoms inside the central core increases the hydrophilicity, as in the 1,4-benzodioxane scaffold of compound **2**; in this case, the presence of a dimethylamine group instead of piperidine one on the lateral chain, contributes to the reduced hydrophobicity of the overall molecule. It should be noted that there is a shortening of the lateral chain of compound **2** when compared to patented compound **1**. This modification should further help in increasing polarity of the new structures, and besides, in Takeda patent, molecules with shorter lateral chain were also reported to be active [6]. Furthermore, we have observed that a shorter lateral chain, with only a methylene, was synthetically more accessible than the ethylene linker.

Another modification concerned the shrinkage of the central bicyclic core, to an indane cycle, leading to compound **3** (Fig. **3**), which lacks one methylene unit when compared to its patented counterparts and, consequently, is less lipophilic. Finally, a non-cyclic scaffold as an isoprenoid moiety, as in compounds **4** and **5** (Fig. **3**), was considered suitable for our goals.

RESULTS AND DISCUSSION

The synthesis of compound **3** has been carried out according to literature and Takeda patent (Scheme **1**). Commercially available 4-benzyloxy-2-hydroxy-benzaldehyde **6** was treated with epichlorohydrin to give aldehyde **7** [8]. A Baeyer-Villiger oxidation converts product **7** to an unstable formate intermediate, that spontaneously produced phenol **8** [9]. Phenol **8** so obtained was cyclized into 1,4-benzodioxane **9** in the presence of KOH 2N [8]. A catalytic reduction with Pd/C 10% yielded compound **10**, bearing a free phenol group. The introduction of a biphenyl substituent (compound **11**) was conducted by reaction with 4-phenylbenzyl chloride in CH_3CN. The subsequent transformation of the primary alcohol into iodide was achieved by reaction with imidazole, iodine and triphenylphospine. This modification allowed us to introduce subsequently a dimethylamino group in order to obtain the desired product **2** [6]. Finally, treatment of compound **2** with $Et_2O{\cdot}HCl$ yielded its hydrochloride salt form **2**·HCl.

Compound **3** was prepared following the procedure described for the patented compound **1**, making some synthetic modifications only where necessary (Scheme **2**). Commercially available 5-methoxy-1-indanone **13** was esterified with $BrCH_2COOEt$ in the presence of LHMDS, to obtain the desired ester **14** in good yield [10]. Product **14** was hydrolyzed with NaOH in EtOH to give carboxylic acid **15**. Then, amide **16** was obtained by reaction of **15** with *N*,*N*-dimethylamine. In the following reduction-elimination reaction, compound **16** was treated with $NaBH_4$ and *p*-TsOH, yielding indene derivative **17**. The double bond was hydrogenated in the presence of Pd/C (compound **18**) and the amide group was reduced with $LiAlH_4$ to obtain the desired amino derivative **19**. Treatment with BBr_3 caused the removal of the *O*-methyl group and subsequent etherification with 4-phenylbenzyl chloride in the presence of NaH produced the final aminoalkyl-substituted indane **3** in good yield.

The synthetic approach exploited for compounds **4** and **5** was based on procedures already developed in our research laboratory. Both isoprenoid molecules were synthesized in six steps, starting from commercially available 3-methyl-2-buten-1-ol **20**, as shown in Scheme **3**. The protection of the hydroxyl group of alcohol **20** was achieved by reaction with 3,4-dihydropyran in the presence of pyridinium *p*-toluensulfonate [11-13], producing a tetrahydropyranyl ether in high yield. Compound **21** was oxidized with *t*-butylhydro-

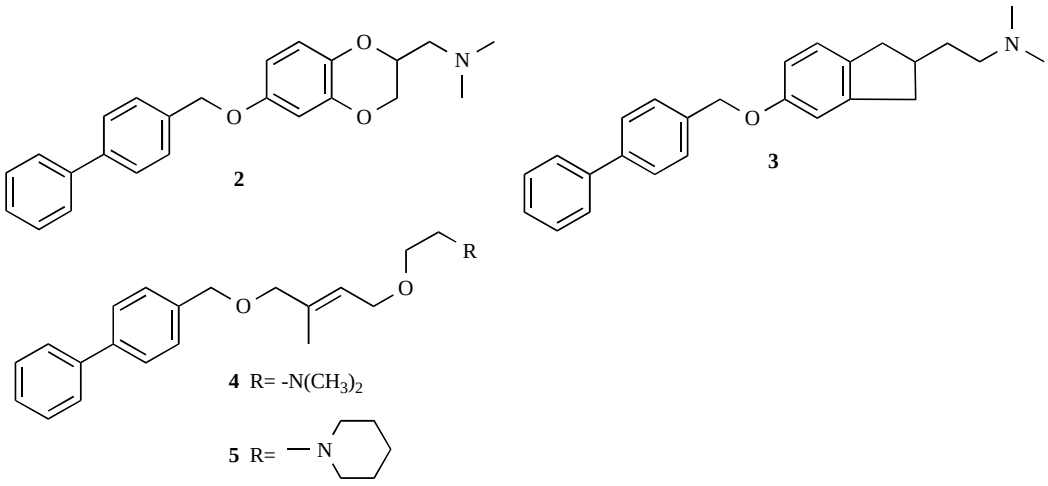
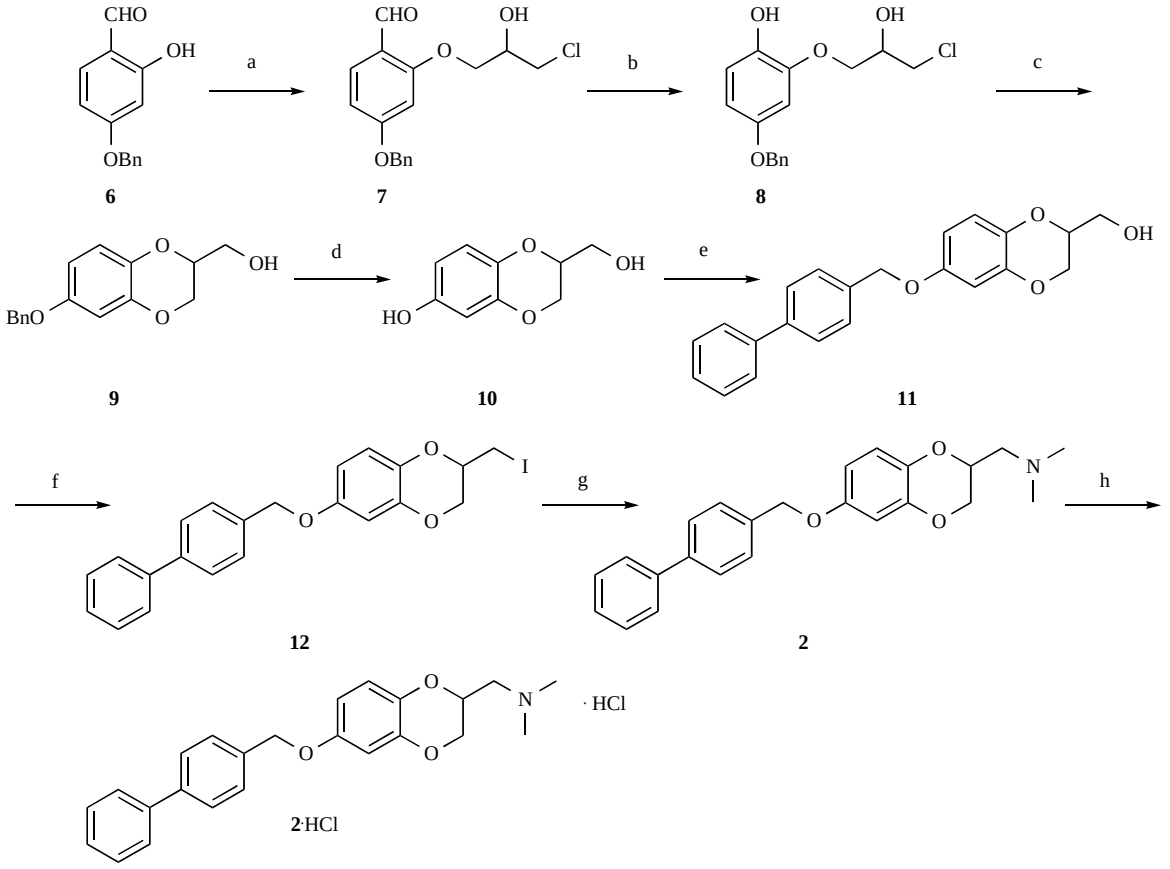
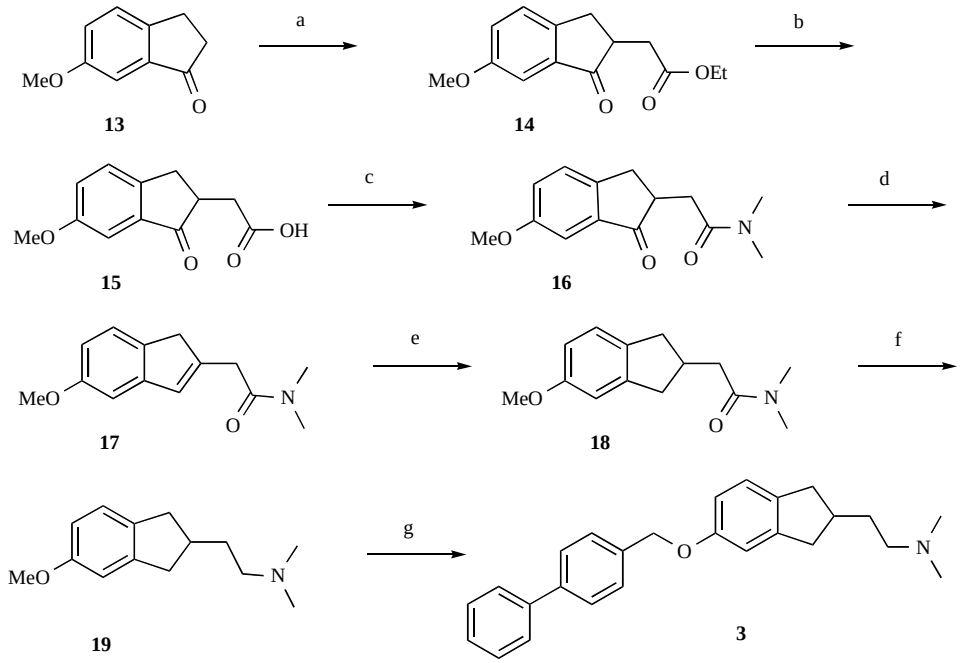


Fig. (3). New structures (**2-5**) inspired to Takeda compounds.



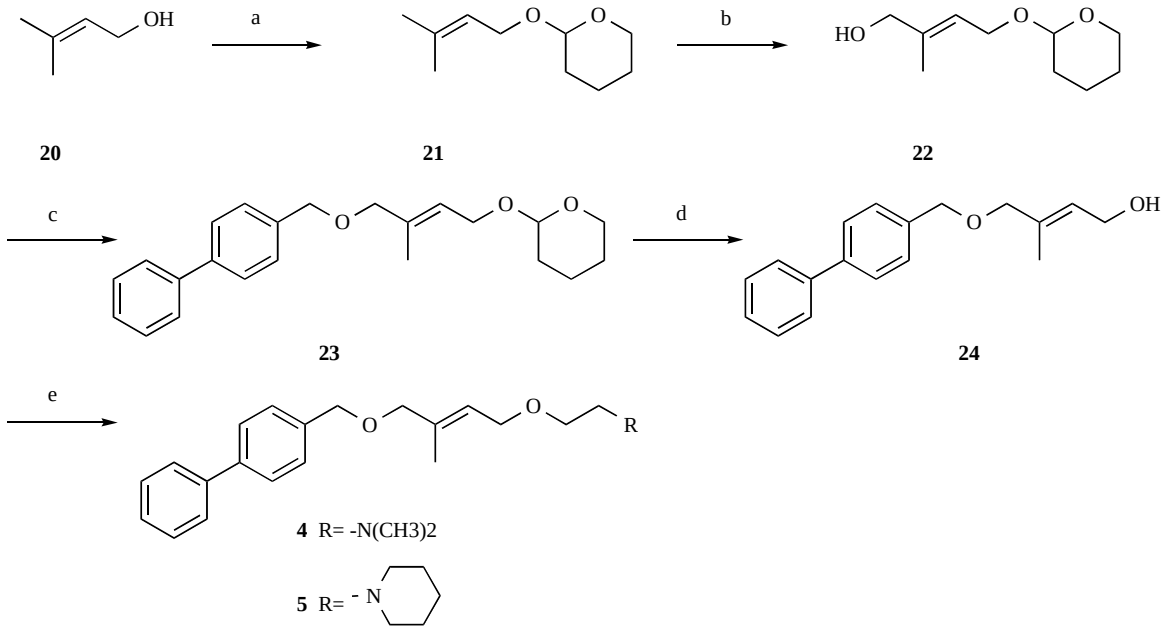
Scheme 1.[a] Synthesis of benzodioxanic compound **2**.

[a]Key: (a) epichlorohydrin, piperidine, 100 °C; (b) H_2O_2 35%, $KHSO_4$, MeOH; (c) KOH 2N; (d) H_2, Pd/C 10%, EtOH-EtOAc; (e) 4-phenylbenzyl-chloride, K_2CO_3, KI, CH_3CN, 60 °C to 80 °C; (f) I_2, Ph_3P, imidazole/THF; (g) dimethylamine, K_2CO_3, I_2, THF; (h) Et_2O·HCl.



Scheme 2.[a] Synthesis of indane compound **3**.

[a]Key: (a) LHMDS, HMPA, $BrCH_2COOEt$, THF, -60 °C; (b) NaOH 2N, EtOH; (c) [3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride, hydroxybenzotriazole, *N*,*N*-dimethylamine, dry DMF, 0 °C; (d) 1) $NaBH_4$, MeOH, 0 °C; 2) dry $PhCH_3$, *p*-TsOH, 120 °C; (e) H_2, Pd/C, EtOH; (f) $LiAlH_4$, dry THF, 70 °C; (g) 1) BBr_3, CH_2Cl_2, -78 °C to 0 °C; 2) NaH, 4-phenylbenzyl chloride, dry DMF, 0 °C to 50 °C, then 0 °C to r. t..



Scheme 3.[a] Synthesis of isoprenoid compounds **4** and **5**.

[a]Key: (a) 3,4-DHP, PPTS, CH_2Cl_2; (b) *t*-BuOOH, H_2SeO_3, salicylic acid; CH_2Cl_2; (c) NaH, 4-phenylbenzyl chloride, dry DMF, 0 °C to 50 °C, then 0 °C to r. t.; (d) PPTS, EtOH, 55 °C; (e) NaH, chloroethyl-*N*,*N*-dimethylamine or *N*-chloroethylpiperidine, dry DMF, 0 °C to 50 °C, then 0 °C to r. t..

peroxide and catalytic H_2SeO_3 in the presence of salicylic acid to give compound **22**, which was then submitted to deprotonation by NaH and, subsequently, to a reaction with 4-phenylbenzyl chloride in DMF, to obtain compound **23**. After deprotection of **23** with pyridinium *p*-toluensulfonate in EtOH, the resulting alcohol **24** was treated with chloroethyl-*N*,*N*-dimethylamine or *N*-chloroethylpiperidine, affording the two desired final products **4** and **5**, respectively.

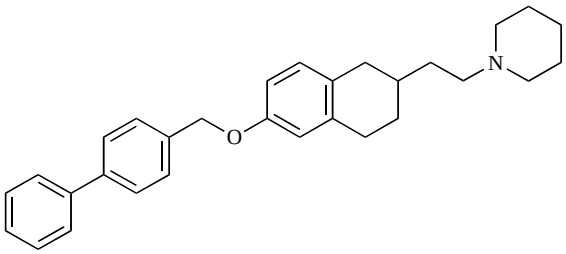
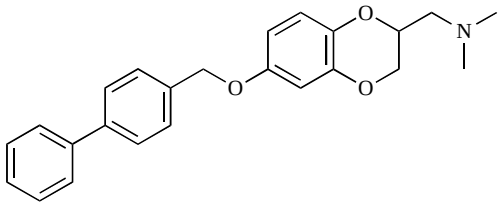
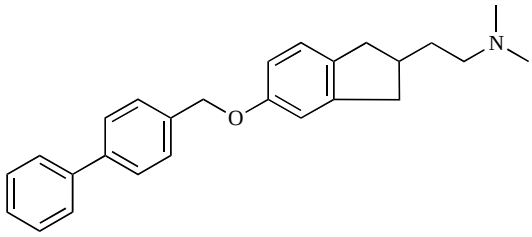
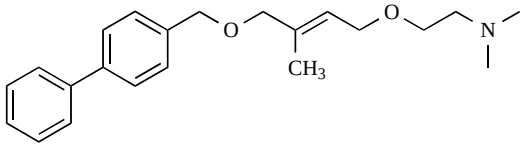
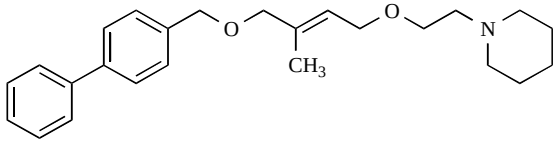
Both Takeda compounds and new derivatives were evaluated at a concentration of 20 μM on BACE1 with a TR-FRET (Time Resolved-Fluorescence Resonance Energy Transfer) assay [14]. IC_{50} values were calculated only for compounds that showed an enzymatic inhibition greater than 50% at 10 μM and are reported in Table **1**. Reference compound **1** was synthesized by following the procedure reported in the Takeda patent [6]. As regards newly synthesized compounds, only the indane analogue **3** showed a notable inhibitory activity in the μM range, possessing an IC_{50} of 12 μM, which is comparable to that of reference compound **1** [15], whereas the other, more polar, derivatives showed an inhibition level lower than 50% at a concentration of 20 μM. These results show that the contraction of the central saturated ring of compound **1** from six to five members and the replacement of the piperidine substituent with a dimethylamino group as in the derivative **3**, led to maintenance of a good level of inhibitory activity on BACE1. On the contrary, the replacement of the bicyclic core with an acyclic isoprenoid moiety, as in compounds **4** and **5**, was detrimental for the activity. A similar decrement was found for compound **2**, suggesting that the introduction of oxygen atoms into the central core and the presence of a methylenic side chain are not suitable strategies to obtain good BACE1 inhibitors.

EXPERIMENTAL

Biological Methods

The time resolved fluorescence quenching assay developed for BACE1 used as substrate a peptide made of 10 amino acids. The sequence contains the KM - NL APP (Amyloid Precursor Protein) Swedish and has a fluorescent Eu^{3+}-chelate coupled to the N-terminus as donor (Ex max 330 nm- Em max 615 nm) and a quenching organic fluorophore at the C-terminus as acceptor. The proteolytic cleavage of the substrate by BACE1 interrupts the energy transfer between donor and acceptor, leading to a linear increase of fluorescence emission at 615 nm of the donor, that is directly related to the enzyme activity and can be followed by kinetic TRF measurements. Problems with compound interference, frequently occurring with fluorescent FRET substrates, are not significant in this assay. A high signal-to-noise ratio of 13 and a Z' factor of 0.76 make this assay well suitable for HTS. The assay was validated by reproducing the IC_{50} values of known and patented inhibitors and by determining the K_i value and binding mechanism of a known Stat-Val peptide inhibitor. All measured IC_{50} values correlated with published data. K_i for the Stat-Val peptide inhibitor was determined with various substrate concentrations (0.1-7 mM) and four different peptide concentrations (6, 15, 30 and 60 nM). Data were plotted with the double reciprocal Lineweaver-Burk plot and K_i was graphically determined through a Cornish-Bowden plot. Reversibility and time dependency were established to better characterize the Stat-Val peptide inhibition. The reversibility was assessed through 10-times dilution of a mixture of 100 ng enzyme and compounds at 10-times the IC_{50} concentration after a pre-incubation of 60 min at 25 °C. The obtained activity was normalized to the control and compared to the activity of the concentrated mixture.

Table 1. BACE1-Inhibitory Activities[a] of Takeda Compound 1 and Derivatives 2-5

Compound	Structure	IC_{50} (μM)
1		11
2		> 20
3		12
4		> 20
5		> 20

[a]Determined by a TR-FRET assay (Time-Resolved Fluorescence Resonance Energy Transfer).

Chemistry

NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063–0.200 mm; Merck) chromatography, or on Isolute pre-packed silica columns and Isolute ionic exchanged pre-packed silica columns as the stationary phase. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualized under a UV lamp and/or with phosphomolybdic acid solution. Evaporation was performed *in vacuo* (rotating evaporator). Sodium sulfate was always used as the drying agent. The organic solvents were dried by distillation from calcium hydride, magnesium methoxide or sodium metal and stored under nitrogen and/or over sodium wire. *N,N*-dimethylformamide (DMF) was purchased from Aldrich in SureSeal containers. Where necessary, reaction requiring anhydrous condition were performed in a flame or oven-dried apparatus under nitrogen atmosphere.

4-(Benzyloxy)-2-(3-chloro-2-hydroxypropoxy)benzaldehyde (7)

Commercially available 4-benzyloxy-2-hydroxy-benzaldehyde **6** (2.00 g, 8.70 mmol) was treated with epichlorohydrin (2.1 mL, 26.1 mmol) and 7-8 drops of piperidine were added. The reaction mixture was stirred for 4 hours at 100 °C in a closed vial. After complete reaction, the reaction mixture was diluted with $CHCl_3$ and concentrated HCl was added to open the unreacted epoxide. The organic phase was separated and washed with water, then evaporated *in vacuo*. The desired product **7** (1.45 g, 52% yield) was purified by flash chromatography (90 to 80 Hexane/EtOAc); ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.77 (dd, 2H, J = 5.2, 1.3 Hz), 4.40-4.00 (m, 3H), 5.13 (s, 2H, benzylic -CH_2-), 6.56 (d, 1H, J = 2.1

Hz), 6.68 (dd, 1H, *J* = 8.6, 2.0 Hz), 7.26-7.50 (m, 5H), 7.77 (d, 1H, *J* = 8.7 Hz), 10.19 (s, 1H, -CHO).

4-(Benzyloxy)-2-(3-chloro-2-hydroxypropoxy)phenol (8)

Aldehyde **7** (1.45 g, 4.54 mmol) was dissolved in MeOH, then H_2O_2 sol. 35% (0.5 ml, 5.90 mmol) and $KHSO_4$ (96 mg, cat) were added. After 1 night at room temperature the mixture was diluted with H_2O and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The crude residue almost entirely constituted by compound **8** was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.71-3.76 (m, 2H), 4.03-4.31 (m, 3H), 4.99 (s, 2H, benzylic -CH_2-), 6.53 (dd, 1H, *J* = 8.6, 2.7 Hz), 6.60 (d, 1H, *J* = 2.7 Hz), 6.85 (d, 1H, *J* = 8.6 Hz), 7.32-7.44 (m, 5H).

(6-(Benzyloxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methanol (9)

Phenol **8** (1.30 g, 4.23 mmol) was treated with an excess of KOH 2N solution. The mixture was allowed to react 1 hour at room temperature and an oil appeared in the mixture. The product was extracted with CH_2Cl_2 and purified by flash chromatography (8:2 Hexane/EtOAc). The purification afforded the desired product **9** as an yellow oil (933 mg, 81% yield); ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.70-3.88 (m, 2H), 3.88-4.40 (m, 3H), 4.98 (s, 2H, benzylic -CH_2-), 6.40-6.60 (m, 2H), 6.80 (d, 1H, *J* = 8.4 Hz), 7.25-7.50 (m, 5H).

2-(Hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-ol (10)

1,4-benzodioxane **9** (887 mg, 3.26 mmol) was dissolved in EtOH (15 mL) and EtOAc (6-7 drops). Pd/C 10% was added and the mixture was allowed to react under H_2 for 5 hours. The mixture was filtered on Celite and the solution was evaporated *in vacuo*, affording pure compound **10** (563 mg, 94% yield); ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.60-4.35 (m, 5H), 6.28-6.44 (m, 2H), 6.75 (d, 1H, *J* = 8.6 Hz).

(6-(Biphenyl-4-ylmethoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methanol (11)

Phenol **10** (563 mg, 3.09 mmol) was dissolved in CH_3CN (5 mL), then K_2CO_3 (768 mg, 5.55 mmol) was added and the mixture was allowed to warm to 60 °C. A solution of 4-phenylbenzyl-chloride (689 mg, 2.81 mmol) in CH_3CN (1 mL) was added. Subsequently, catalytic KI (a spatula tip) was added. After the mixture was stirred at 80 °C for 1 night, CH_3CN was evaporated. The crude residue was acidified with HCl 4N solution and extracted with $CHCl_3$. The desired product **11** (968 mg, 90% yield) was obtained after purification by flash chromatography (90 to 80% Hexane/EtOAc); ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.70-3.98 (m, 2H), 3.98-4.40 (m, 3H), 5.02 (s, 2H, benzylic -CH_2-), 6.43-6.62 (m, 2H), 6.82 (d, 1H, *J* = 8.6 Hz), 7.25-7.70 (m, 9H).

6-(Biphenyl-4-ylmethoxy)-2-(iodomethyl)-2,3-dihydrobenzo[b][1,4]dioxine (12)

To a solution of Ph_3P (88.1 mg, 0.518 mmol) in THF (1.5 mL), imidazole (21.8 mg, 0.320 mmol) and I_2 (85.2 mg, 0.336 mmol) were added. Then, a solution of alcohol **11** (200 mg, 0.574 mmol) in THF (1.5 mL) was added. After 5 hours at room temperature, the mixture was treated with $Na_2S_2O_3$ saturated solution and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The crude residue almost entirely constituted by compound **12** was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.31-3.34 (m, 2H), 4.09-4.39 (m, 3H), 5.02 (s, 2H, benzylic -CH_2-), 6.50-6.56 (m, 2H), 6.79-6.84 (m, 1H), 7.34-7.73 (m, 9H).

1-(6-(Biphenyl-4-ylmethoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-N,N-dimethylmethanamine (2)

To a solution of compound **12** (261 mg, 0.569 mmol) and dimethylamine (3 mL, 4.55 mmol) in THF (1 mL), K_2CO_3 (197 mg, 1.42 mmol) and a solution of I_2 (250 mg, 0.985 mmol) in THF (2.5 mL) were added. The mixture was stirred at room temperature for 3 days. The mixture was washed with H_2O and extracted with CH_2Cl_2. Purification by flash chromatography (80 to 50% Hexane/EtOAc) afforded the final product **2** as a white solid, which has been treated with HCl to form the hydrochloride salt **2·HCl** as a white solid (39.0 mg, 17% yield); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.33 (s, 6H, -$N(CH_3)_2$), 2.40-2.70 (m, 2H), 3.86-4.04 (m, 1H), 4.14-4.36 (m, 2H), 5.02 (s, 2H, benzylic -CH_2-), 6.46-6.60 (m, 2H), 6.83 (d, 1H, *J* = 8.4 Hz), 7.80-7.26 (m, 9H).

Ethyl 2-(6-methoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)acetate (14)

A solution of the commercial available 6-methoxy-2,3-dihydro-1H-inden-1-one **13** (1.0 eq) in a minimal amount of THF was added dropwise to a stirred solution of LHMDS (1.2 eq) at -60 °C. After 30 minutes, HMPA (1.2 eq) and $BrCH_2COOEt$ (2.0 eq) were added and the reaction was left under stirring for 1 h at -60 °C. After TLC analysis, the mixture was stopped: NH_4Cl solution was added and the organic layer extracted with AcOEt, dried over Na_2SO_4 and concentrated. The desired product was purified with silica and ionic exchange pre-packed cartridges (yield 70%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.22 (t, 3H, *J* = 7.1 Hz, -C(=O)$OCH_2C\underline{H_3}$), 2.66 (dd, 1H, *J* = 16.8, 8.8 Hz), 2.87 (dd, 1H, *J* = 15.2, 3.9 Hz), 2.71-3.09 (m, 2H), 3.38 (dd, 1H, *J* = 16.6, 7.6 Hz), 3.83 (s, 3H, -OCH_3), 4.13 (q, 2H, *J* = 7.1 Hz, -C(=O)$OC\underline{H_2}CH_3$), 716-7.37 (m, 3H).

2-(6-Methoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)acetic acid (15)

A solution of NaOH 2N (10 eq) was added to a solution of compound **14** (1.0 eq) in a minimal amount of EtOH and the resulting mixture was allowed to react for 5 h at room temperature. The reaction was stopped by adding HCl 10% solution and then the organic layer was extracted with Et_2O, dried over Na_2SO_4 and concentrated. The crude residue was purified with silica and ionic exchange pre-packed cartridges to obtain the desired acid **15** (yield 97%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.64 (dd, 1H, *J* = 17.8, 9.4 Hz), 2.81 (dd, 1H, *J* = 16.7, 3.9 Hz), 2.97-3.10 (m, 2H), 3.42 (dd, 1H, *J* = 16.6, 7.5 Hz), 3.84 (s, 3H, -OCH_3), 7.15-7.38 (m, 3H).

2-(6-Methoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)-N,N-dimethylacetamide (16)

To a cooled (0 °C) and stirred solution of carboxylic acid **15** (1.0 eq) in dry DMF, 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride (1.1 eq), hydroxybenzotriazole (1.0 eq) and *N*,*N*-dimethylamine (1.1 eq) were added. The solution was stirred for 2 h. After evaporation of the

solvent, the residue was poured into water and extracted with AcOEt. The extracts were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography with AcOEt as eluent (yield 40%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.63 (dd, 1H, *J* = 17.1, 9.2 Hz), 2.81 (dd, 1H, *J* = 16.7, 3.9 Hz), 2.94 (s, 3H), 3.02 (s, 3H), 2.97-3.09 (m, 2H), 3.41 (dd, 1H, *J* = 16.7, 7.4 Hz), 3.83 (s, 3H, -OCH_3), 7.14-7.21 (m, 2H), 7.33 (d, 1H, *J* = 8.2 Hz).

2-(5-Methoxy-1H-inden-2-yl)-N,N-dimethylacetamide (17)

To a cooled (0 °C) and stirred solution of amide **16** (1 eq) in a minimal amount of MeOH, $NaBH_4$ (2.5 eq) was added dropwise and the resulting solution was stirred for 1 h. After evaporation of the solvent, the residue was neutralized with HCl 1N, extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated. The residue was diluted in dry toluene and *p*-TsOH (cat) was added. The reaction mixture was heated to reflux at 120 °C and stirred for 1 h . After TLC analysis, the reaction was stopped by adding $NaHCO_3$ sat. solution and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude residue was used for the next step without further purification (yield 65%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.98 (s, 3H), 3.05 (s, 3H), 3.37 (s, 2H), 3.57 (s, 2H), 3.81 (s, 3H,-OCH_3), 6.69 (dd, 1H, *J* = 8.1, 2.4 Hz), 6.85-6.99 (m, 1H); 7.14-7.28 (m, 2H).

2-(5-Methoxy-2,3-dihydro-1H-inden-2-yl)-N,N-dimethylacetamide (18)

To a stirred solution of amide **17** (1.0 eq) in a minimal amount of EtOH, Pd/C (0.1 eq) was added, under vacuum and N_2. The mixture was allowed to react under H_2 for 13 h. After TLC analysis, the desired product was collected by filtration (yield 76%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.46-2.69 (m, 4H), 2.96 (s, 3H), 2.98 (s, 3H), 2.83-3.21 (m, 3H), 3.77 (s, 3H, -OCH_3), 6.66-6.78 (m, 2H), 7.08 (d, 1H, *J* = 8.0 Hz).

2-(5-Methoxy-2,3-dihydro-1H-inden-2-yl)-N,N-dimethylethanamine (19)

To a stirred solution of amide **18** (1.0 eq) in dry THF, $LiAlH_4$ (1.5 eq) was added and the mixture was heated at 70 °C for 1 h. After TLC analysis, water and NaOH 1N were added, then the organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica and ionic exchange pre-packed cartridges (yield 63%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.65-1.76 (m, 2H), 2.29 (s, 6H, -$N(CH_3)_2$), 2.37-2.63 (m, 4H), 2.68-2.84 (m, 1H), 2.92-3.07 (m, 2H), 3.77 (s, 3H, -OCH_3), 6.64-6.73 (m, 2H), 7.06 (d, 1H, *J* = 8.0 Hz).

2-(5-(Biphenyl-4-ylmethoxy)-2,3-dihydro-1H-inden-2-yl)-N,N-dimethylethanamine (3)

A solution of BBr_3 1M in CH_2Cl_2 (3 eq) was added dropwise at -78 °C to a stirred solution of amine **19** (1.0 eq) in CH_2Cl_2. After 30 minutes, the mixture was allowed to warm to room temperature and left stirring for 1 h. After TLC analysis, $NaHCO_3$ sat. solution was added and the organic layer was extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated. The residue was purified by SCX pre-packed cartridges. To a stirred solution of alcohol resulting from the previous step (1 eq) in dry DMF, at 0 °C, NaH (60% oil dispersion, 2.5 eq) was added and the mixture was stirred at 50 °C for 1 h. The mixture was then cooled to 0 °C, 4-phenylbenzyl chloride (1.1 eq) was added and the resulting mixture was stirred at room temperature for 2 h. After evaporation of the solvent, the residue was diluted with water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated, affording a crude residue that was purified by silica and ionic exchange pre-packed cartridges. Yield 41%, ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.46-1.84 (m, 2H), 2.36 (s, 6H, -$N(CH_3)_2$), 2.42-2.66 (m, 5H), 2.90-3.10 (m, 2H), 5.07 (s, 2H, benzylic -CH_2-), 6.74-6.88 (m, 2H), 7.08 (d, 1H, *J* = 8.4 Hz), 7.34-7.63 (m, 9H).

2-(3-methylbut-2-enyloxy)tetrahydro-2H-pyran (21)

To a solution of the commercial available 3-methyl-2-buten-1-ol (1.0 eq) in a minimal amount of CH_2Cl_2, 3,4-dihydropyrane (1.5 eq) and PPTs (0.10 eq) were added and the mixture was stirred for 4 h at room temperature. After TLC analysis, the reaction mixture was partially evaporated, diluted with Et_2O and washed with $NaHCO_3$ sat. solution. The organic layer was dried over Na_2SO_4 and concentrated affording the desired product, that was purified with silica anionic exchange pre-packed cartridges (yield 87%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.40-1.85 (m, 6H), 1.64 (s, 3H), 1.70 (s, 3H), 3.40-3.51 (m, 1H), 3.80-3.86 (m, 1H), 3.92 (dd, 1H, *J* = 12.1, 6.0 Hz), 4.17 (dd, 1H, *J* = 12.1, 6.0 Hz), 4.58 (t, 1H, *J* = 4.0 Hz), 5.28 (t, 1H, *J* = 6.0 Hz).

(E)-2-methyl-4-(tetrahydro-2H-pyran-2-yloxy)but-2-en-1-ol (22)

A solution of compound **21** (1.0 eq) and *t*-butylhydroperoxide (3.2 eq) in CH_2Cl_2 was treated with H_2SeO_3 (0.11 eq) and salicylic acid (0.11 eq) and the reaction mixture was stirred for 14 h at room temperature. The solvent was removed *in vacuo* and *t*-butylhydroperoxide was removed by washing repeatedly with toluene. The residue was dissolved in Et_2O, washed with $NaHCO_3$ sat. solution to remove H_2SeO_3, dried over Na_2SO_4 and concentrated. The residue was purified by silica and ionic exchange pre-packed cartridges (yield 40%); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.54-1.85 (m, 9H), 3.47-3.57 (m, 1H), 3.83-3.94 (m, 1H), 4.01-4.10 (m, 4H), 4.29 (dd, 1H, *J* = 12.1, 6.4 Hz), 5.63 (t, 1H, *J* = 6.1 Hz).

(E)-2-(4-(biphenyl-4-ylmethoxy)-3-methylbut-2-enyloxy)tetrahydro-2H-pyran (23)

Alcohol **22** (1.0 eq) was dissolved in dry DMF and cooled to 0 °C prior to NaH (60% oil dispersion, 2.5 eq) addition, then the mixture was stirred at 50 °C for 1 h. Then, the mixture was cooled at 0 °C and 4-phenylbenzyl chloride (1.1 eq) was added. The resulting mixture was stirred for 24 h at room temperature, then poured into water and extracted with AcOEt. The combined extracts were dried over Na_2SO_4 and concentrated (yield 30%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.70-1.82 (m, 9H), 3.48-3.54 (m, 1H), 3.80-3.90 (m, 1H), 3.98 (s, 2H), 4.10-4.20 (m, 2H), 4.52 (s, 2H, benzylic -CH_2-), 4.60 (dd, 1H, *J* = 14.8, 2.9 Hz), 5.66-5.80 (m, 1H), 7.33-7.48 (m, 5H), 7.55-7.62 (m, 4H).

(E)-4-(biphenyl-4-ylmethoxy)-3-methylbut-2-en-1-ol (24)

To a solution of compound **23** (1.0 eq) in a minimal amount of EtOH, PPTs (0.10 eq) was added and the reaction mixture stirred at 55 °C for 6 h. The solvent was partially evaporated *in vacuo* and the resulting solution was diluted

with Et_2O and washed with half-saturated brine to remove the catalyst. The organic layer was dried over Na_2SO_4 and concentrated affording a crude residue that was purified with silica and ionic exchange pre-packed cartridges (yield 62%); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.72 (d, 3H, *J* = 7.3 Hz), 3.87 (d, 2H, *J* = 18.0 Hz), 4.18 (dd, 2H, *J* = 24.1, 6.5 Hz), 4.55 (s, 2H, benzylic -CH_2-), 5.60-5.80 (m, 1H), 7.31-7.48 (m, 5H), 7.56-7.61 (m, 4H).

(E)-2-(4-(Biphenyl-4-ylmethoxy)-3-methylbut-2-enyloxy)-N,N-dimethylethanamine (4), (E)-1-(2-(4-(biphenyl-4-ylmethoxy)-3-methylbut-2-enyloxy)ethyl)piperidine (5)

Alcohol **24** (1.0 eq) was dissolved in dry DMF and cooled to 0 °C prior to NaH (60% oil dispersion, 2.5 eq) addition. The mixture was stirred at 50 °C for 1 h. Then, after cooling to 0 °C, chloroethylamine (1.1 eq) was added and the resulting mixture was stirred for 18-24 h at room temperature. Then, the reaction mixture was poured into water and extracted with AcOEt. The combined extracts were dried over Na_2SO_4 and concentrated, then purified with silica and ionic exchange pre-packed cartridges (95:5 CH_2Cl_2/MeOH); **4**: Yield 30%, ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.70 (d, 3H, *J* = 10.2 Hz), 2.3 (s, 6H, -$N(CH_3)_2$), 2.52-2.64 (m, 2H), 3.47-3.64 (m, 2H), 3.94 (d, 2H, *J* = 9.1 Hz), 4.07-4.13 (m, 2H), 4.57 (s, 2H, benzylic -CH_2-), 5.64-5.78 (m, 1H), 7.36-7.48 (m, 5H), 7.55-7.61 (m, 4H); **5**: Yield 25%, ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.20-1.66 (m, 6H), 1.70 (d, 3H, *J* = 11.3 Hz), 2.45 (m, 4H), 2.57 (t, 2H, *J* = 5.9 Hz), 3.50-3.68 (m, 2H), 3.93 (d, 2H, *J* = 11.1 Hz), 4.10 (t, 2H, *J* = 6.5 Hz), 4.56 (s, 2H, benzylic -CH_2-), 5.64-5.74 (m, 1H), 7.33-7.48 (m, 5H), 7.55-7.61 (m, 4H).

CONCLUSION

There are many evidences that BACE1 inhibitors prevent the formation of Aβ plaques, so they have the potential to be efficacious agents in the treatment of Alzheimer's disease. In this work, we reported the design, synthesis and biological evaluation of a series of BACE1 inhibitors, using tetraline derivatives patented by Takeda as a starting point. One of these patented compounds, the piperidine-substituted derivative **1**, showed a good inhibitory activity, with an IC_{50} in the micromolar range, but it possesses an high degree of lipophilicity, resulting in diminished drug-like properties. Depending on these features, our strategy consisted in replacing the common tetralinic core with more polar scaffolds, keeping the biphenyl group and the amino side-chain constant. Among the new synthesized molecules, indane derivative **3** showed a good IC_{50} value, comparable to that of reference compound **1**, demonstrating that the contraction of the saturated ring of the tetraline moiety from six to five atoms is a good structural modification for maintaining a good BACE1 inhibitory potency and, at the same time, for enhancing the polarity of the structure. On the contrary, other more polar analogues (**2**, **4**, and **5**) did not reach a 50% inhibition at 20 μM. These findings may constitute a basis for the development of more potent BACE1 inhibitors possessing better physico-chemical properties and, consequently, a higher degree of drug-likeness.

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ABBREVIATIONS

BACE1 = β-secretase or β-site APP cleaving enzyme

AD = Alzheimer's disease

Aβ = amyloid β

TR-FRET = Time Resolved-Fluorescence Resonance Energy Transfer

REFERENCES AND NOTES

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[15] In our hands, reference compound **1** exhibited a weaker inhibitory activity (IC_{50} = 11 μM) than that previously reported (IC_{50} = 0.35 μM) in ref.[6]. This may be due to the differences in experimental methods utilized for this determination.