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BACE1 inhibitory activities of enantiomerically pure, variously substituted *N*-(3-(4-benzhydrylpiperazin-1-yl)-2-hydroxypropyl) arylsulfonamides

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ABSTRACT

β-Secretase (BACE1) has been widely recognized as one of the possible therapeutic targets for the treatment of Alzheimer's disease. In this paper, we report the synthesis and the BACE1 inhibitory activity of new, variously substituted *N*-(3-(4-benzhydrylpiperazin-1-yl)-2-hydroxypropyl) arylsulfonamides. Each enantiomeric form was separately evaluated in BACE1 inhibition assays and IC_{50} values were obtained in the low micromolar range. According to our biological results and docking studies, it can be asserted that the stereochemistry around the OH group in the central hydroxyethylamino linker does not significantly influence the BACE1 inhibitory activity of this type of molecules.

1. Introduction

Alzheimer's disease (AD) is the most common form of senile dementia and is characterized by a neurodegenerative disorder that causes a progressive damage to the cholinergic and dopaminergic systems responsible for memory, learning and behaviour. In the brain neurons of AD patients there are relevant amounts of extracellular senile plaques, mainly constituted by amyloid β (Aβ) peptide aggregates[1] and intracellular neurofibrillary tangles made of tau proteins,[2] both of which play a possible role in the progression of the pathology.[3]

The Aβ peptide is generated from proteolytic processing of the amyloid precursor protein (APP) first by β-secretase (BACE1) followed by γ-secretase. BACE1 catalyses the first rate-limiting step of Aβ production. Furthermore, it has been discovered that BACE1 knockout (BACE1 −/−) mice are devoid of the ability to generate Aβ, but they are healthy.[4] It is therefore considered that BACE1 inhibition will be well tolerated.

Therefore, BACE1 is now considered as the preferred therapeutic target for lowering brain Aβ levels in the treatment or prevention of AD.[5]

The identification of this promising pharmacological target has so far led to the development of selective BACE1 inhibitors, many of which incorporate the hydroxyethylamine transition state isostere moiety (HEA)[6] and have a peptidomimetic structure.

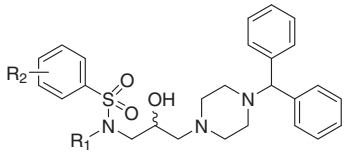

Figure 1. General structure of chiral *N*-(3-(4-benzhydrylpiperazin-1-yl)-2-hydroxypropyl) arylsulfonamides (**1**–**4a**,**b**; see Table 1).

In this paper, we report the synthesis and the BACE1 inhibitory activity of new non-peptidomimetic derivatives characterized by a N-substituted arylsulfonamido moiety linked, through a 2-hydroxypropyl portion, to a benzhydryl-piperazine (Fig. 1).

Although an hydroxyethylamino scaffold is structurally recognisable, these type of compounds cannot be considered as 'classical' hydroxyethylamine (HEA) isosteres, because of the alkylation of both nitrogen-atoms of the central linker. To evaluate if and how the stereochemistry around the OH group could influence the BACE1 inhibitory activity, each new compound was synthesized and assayed in both enantiomeric forms, starting from optically pure precursors.

2. Results and discussion

Compounds **1a**,**b**, **2a**,**b**, **3a**,**b** and **4a**,**b** were synthesized from commercially available 1-benzhydryl-piperazine **5**, as outlined in

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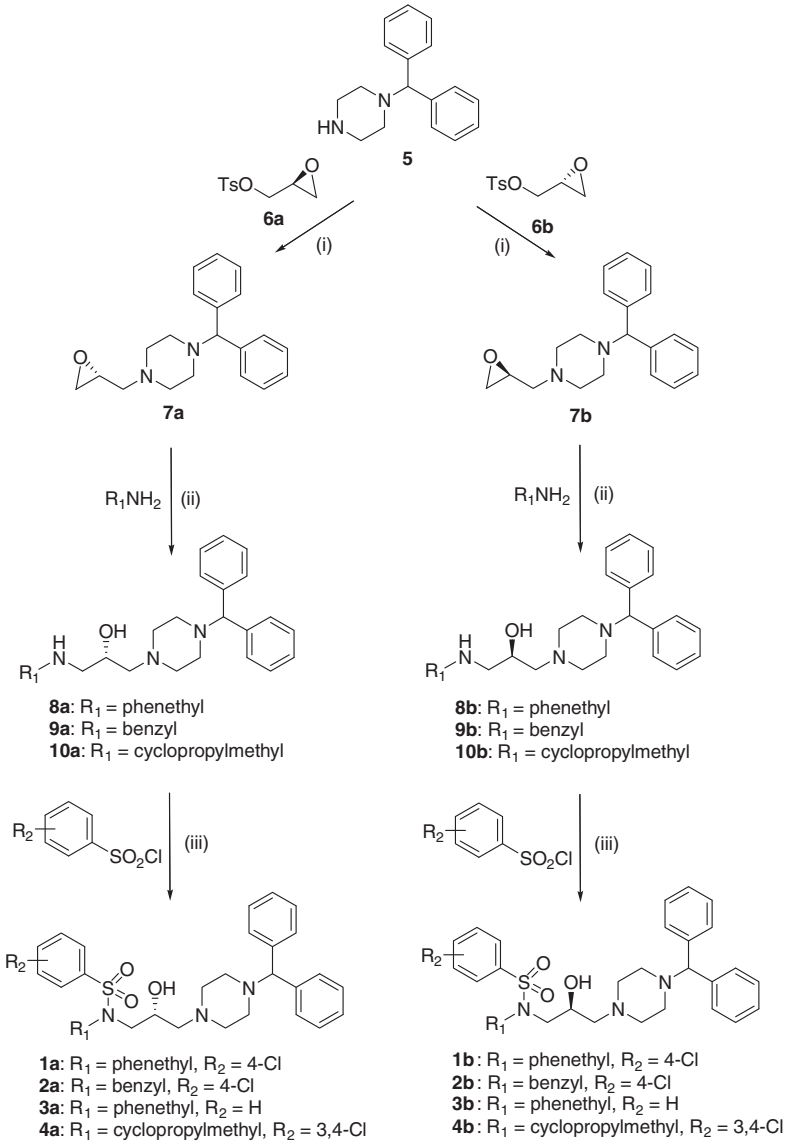
Scheme 1. Reaction of enantiomerically pure (*S*)-glycidyl tosylate **6a** or (*R*)-glycidyl tosylate **6b** with 1-benzhydryl-piperazine **5**, in the presence of K_2CO_3 in DMSO, yielded epoxide intermediates **7a** and **7b**, respectively. These were treated directly with phenethyl-, benzyl-, or cyclopropylmethyl-amine in ethanol, affording intermediates **8a,b**, **9a,b** and **10a,b**. Reaction of these amines with the appropriately substituted phenylsulfonyl chloride in DCM, using catalytic amounts of DMAP, yielded final products **1a,b**, **2a,b**, **3a,b** and **4a,b**.

BACE1 inhibitory activities of the newly synthesized compounds were determined by a previously reported fluorescence-based assay[7] and the results are reported in Table 1.

The predicted in silico Log BB values (Table 1),[8–11] defined as the ratio of the steady-state concentrations of a compound in the brain to those in the blood (Log BB = Log [brain]/[blood]), are quite favourable for the whole series, with values ranging from −0.5 to +0.1, with negligible differences between the enantiomeric forms. Therefore, these derivatives are predicted to be able to efficiently cross the blood-brain barrier and then reach satisfactory concentrations in the central nervous system, comparable to those present in the blood stream.

Enantiomers **1a** and **1b**, possessing a *p*-chlorophenyl-(*N*-phenethyl)-sulphonamido moiety, are the most potent inhibitors of the series, with IC_{50} values of, respectively, 2.3 and 1.9 μM. Substitution of the phenethyl group (R_1) with a benzyl group (maintaining R_2 = 4-Cl), as in compounds **2a** and **2b**, or the replacement of the chlorine atom (R_2) in the *p*-chlorophenyl-sulphonamido moiety with an hydrogen (maintaining R_1 = phenetyl), as in **3a** and **3b**, results in about a twofold decrease of BACE1 inhibition. Likewise, the simultaneous introduction of a 3,4-chloro substituent (R_2) and of a *N*-cyclopropylmethyl group (R_1), as in compounds **4a** and **4b**, results again in a significant drop of activity. Finally, for each couple of enantiomers, the IC_{50} values of *R*- and *S*-isomers are approximately the same, suggesting that the stereochemistry around the OH group does not significantly influence the BACE1 inhibitory activity of this type of molecules (indeed, this was previously hypothesized by Fujimoto et al. for related HEA-inhibitors[12]). However, it is important to underline that in previous works on hydroxyethylamine-based aspartyl protease inhibitors, the hydroxyl stereochemistry was found to be tremendously important, leading oftentimes to as much as 1000-fold difference.[6b]



Scheme 1. Reagents and conditions: (i) K_2CO_3, DMSO; (ii) EtOH; (iii) CH_2Cl_2, DMAP, pyridine.

Table 1
Structures, BACE1 inhibitory activity and predicted Log BB values of benzhydryl-piperazines **1a**,**b**, **2a**,**b**, **3a**,**b** and **4a**,**b**

Compd	R_1	R_2	Stereo chemistry	IC_{50}[a] (μM)	Log BB_{pred}[b]
1a	Phenethyl	4-Cl	R	2.3	−0.2
1b	Phenethyl	4-Cl	S	1.9	−0.3
2a	Benzyl	4-Cl	R	6.3	0
2b	Benzyl	4-Cl	S	5.4	+0.1
3a	Phenethyl	H	R	5.1	−0.3
3b	Phenethyl	H	S	6.9	−0.5
4a	Cyclopropylmethyl	3,4-Cl	R	5.4	0
4b	Cyclopropylmethyl	3,4-Cl	S	4.7	−0.1

[a] IC_{50} measurements were performed as reported in Ref. 7. Data represent mean values for at least three separate experiments. Standard errors are not shown for the sake of clarity and were never higher than 15% of the means.
[b] Predicted blood-brain barrier permeation (Log BB = Log [brain]/[blood]).[8]

The most active compounds (**1a** and **1b**), have been submitted to molecular docking studies in an attempt to understand the interactions of these molecules with the catalytic site of BACE1 (Figs. 2 and 3). The computational protocol used in this analysis has been recently exploited with promising results for the evaluation of α-naphthylaminopropan-2-ol derivatives.[15]

As we can see from the superimpositions of inhibitors **1a** and **1b** (in cyan and magenta, respectively, Fig. 2), the binding modes of the two enantiomeric forms are comparable. In particular, both *R* and *S* enantiomers are able to create a strong hydrogen bond with only one of the two catalytic aspartic acids, Asp228. These results are in agreement with the very similar inhibitory activity values found with both the enantiomers. Consequently, it is deducible that the chirality of the *C*–OH in the hydroxyethylamine isoster portion of this kind of structures does not have any significant influence on the BACE1 inhibitory activity. In details (Fig. 3), the two rings of the benzhydryl-moiety are respectively placed into the S2-pocket (surrounded by Gly230, Thr231 and Thr232), and S3-pocket (Ile230). The piperazine portion lies on the S1-pocket, interacting with Tyr51 and Phe108. The S2′-pocket host a big part of the sulfonamido-terminal portion of the inhibitor. In fact, the aromatic ring of the phenylethyl-substituent interacts with Val69 and Ile126, whereas other hydrophobic interactions occur between the *p*-chlorophenyl ring and Ile226, Thr329, and Val332. The S2′-pocket delimitation is completed by Ser35, Gly34, Tyr71 and, in particular, by Tyr198 which closely interacts with one oxygen atom of the sulfonyl portion by means of a hydrogen bond through its phenol OH group. Finally, the secondary alcohol present on the chiral carbon participates to a hydrogen bond network with Asp228 and Arg235. In this binding orientation the protonation of the piperazine seems to have a marginal effect on the ligand–protein interaction. Indeed the nearer residue is the oxygen of the backbone of Gly230 at a distance of about 4 Å from the protonated nitrogen. Furthermore they are not correctly oriented to create a strong interaction.

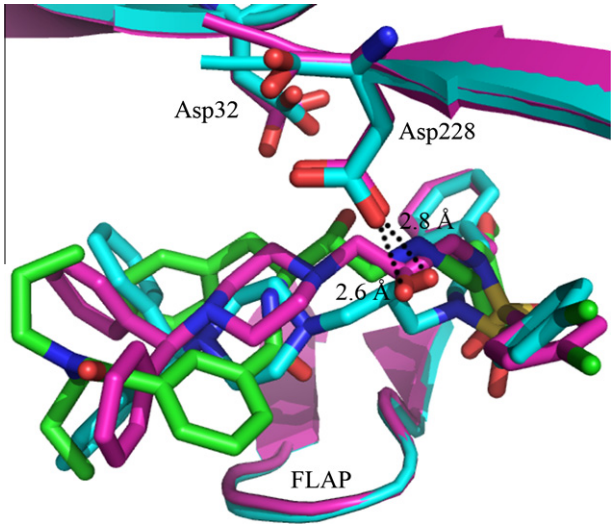


Figure 2. Superimposition of Induce Fit[13] docking poses for inhibitors **1a** (cyan) and **1b** (magenta). The molecule co-crystalized with the BACE1 X-ray crystal structure (PDB code 1W51[14]) used in this study is shown as reference in green. BACE1 active site is shown and the FLAP region is highlighted together with the two catalytic aspartic acids (Asp32 and Asp228).

The comparison of the predicted binding mode with a standard orientation of a HEA inhibitor (Fig. 3) points out the peculiarities of the scaffold in study. Even if there are common steric and electrostatic similarities to the known HEA ligands the resulting most promising pose for this new class of inhibitors can be considered 'upside-down' to the reference. Principally for steric reasons this class of inhibitors seems to not adopt the standard HEA binding mode. The principal reason is the bulky N-substituted arylsulphonylamino moiety that in the proposed binding mode creates good van der Waals interactions, while in the standard HEA orientation it would result in not favourable interactions or steric clashes with the protein. However further studies are needed to validate this preliminary hypothesis. In case of a confirmation of this ligand–protein complex conformation, this study will represent an important starting point to optimize the lead to fully exploit its peculiar binding mode.

3. Conclusion

In conclusion, we have synthesized a new series of chiral BACE1 inhibitors possessing a *N*-(3-(4-benzhydrylpiperazin-1-yl)-2-hydroxypropyl)arylsulfonamido structure. The most active enantiomeric compounds **1a** and **1b** showed promising IC_{50} values (2.3 and 1.9 μM, respectively). Some derivatives are predicted to be able to cross the blood-brain barrier, suggesting that they may reach and inhibit BACE1, inside the central nervous system. According to our biological results and docking studies, it can be asserted that the BACE1 inhibitory activity of this type of compounds does not show a significant dependency upon the absolute configuration of the chiral carbon in the central hydroxyethylamino linker.

4. Experimental section

Purity of the final products was determined by HLPC technique. Preparative HLPC was run using a Waters 2767 system with a binary Gradient Module Waters 2525 pump and coupled to a Waters Micromass ZQ (ES) or Waters 2487 DAD, using a Supelco Discovery HS C18 5.0 μm 10 × 21.2 mm column. Gradients were run using 0.1% formic acid/water and 0.1% formic acid/acetonitrile with gradient 5:95–95:5. In all cases, purity of analyzed compounds was ⩾98%. The characterization by mass spectrometry was carried out by a Perkin

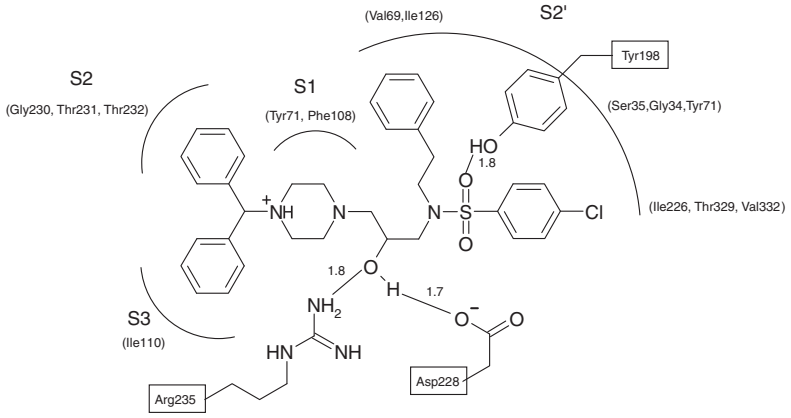


Figure 3. Principal interactions scheme of **1b** with BACE1 active pocket as resulting from the docking study. Hydrogen bonding are shown as dotted lines, important distances in Å are quoted, and the principal amino acids creating van der Waals interactions with the inhibitors for every active sitesubpocket are shown in parentheses.

Elmer/Sciex (Concord, ON, Canada) API 365 triple quadrupole mass spectrometer, equipped with a Turbo ionspray ion source and coupled to a Perkin Elmer (Waltham, MA, USA) 200 Series HPLC system, including a binary micro pump system, an high pressure mixer, and an autosampler. Experiments were performed either in full scan mode, to get the molecular weight, or in Product ion scan mode (MS–MS), to obtain structural information. Analytical data are shown for only one enantiomer of each couple. Chiral HPLC analysis: Analytical HPLC were performed on a Waters 600E equipped with a Varian Prostar 325 detector with detection at 254 nm and 220 nm. The stationary phase used was an analytical chiral column (Chiralcel OD-H column, Daicel Chemical, Tokyo, Japan). HPLC grade 2-propanol and *n*-hexane were purchased from Sigma–Aldrich and used as received. The mobile phase was in all cases *n*-hexane/2-propanol (75:25), with a flow rate of 0.5 mL/min.

4.1. 1-Benzhydryl-4-(*S*)-1-oxiranylmethyl-piperazine (7b)

To a solution of 1-benzhydryl-piperazine (**5**) (4.20 g, 16.64 mmol) in DMSO (30 mL), K_2CO_3 (2.99 g, 21.64 mmol) and toluene-4-sulfonic acid (*R*)-1-oxiranylmethyl ester (**6b**) (3.80 g, 16.64 mmol) were added. The mixture was stirred at room temperature for 24 h, poured into water (300 mL) and extracted with EtOAc (3 × 200 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain a residue that was purified by flash chromatography (eluent: EtOAc/CH_2Cl_2 8:2) to afford 1.81 g of the title compound (yield: 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm): 2.32 (dd, 1H, J = 13.2, 6.8 Hz), 2.35–2.65 (m, 8H), 2.47 (dd, 1H, J = 5.2, 2.8 Hz), 2.71 (dd, 1H, J = 13.2, 3.6 Hz), 2.76 (t, 1H, J = 4.6 Hz), 3.05–3.12 (m, 1H), 4.23 (s, 1H), 7.15–7.19 (m, 2H), 7.24–7.28 (m, 4H), 7.40–7.42 (m, 4H). MS: the experiment in full scan mode showed a pseudo-molecular ion ([M+H]$^+$) at *m*/*z* 309 Th; MS–MS of the ion at *m*/*z* 309 Th (20 eV) provided a spectrum constituted by three main ions: *m*/*z* 167 (benzhydryl$^+$, 100), 309 (0.9), 141 (M$^+$-benzhydryl, 1.2).

4.2. 1-Benzhydryl-4-(*R*)-1-oxiranylmethyl-piperazine (7a)

Same procedure as described above starting from toluene-4-sulfonic acid (*S*)-1-oxiranylmethyl ester (**6a**), to obtain 2.03 g of the title compound (yield: 40%). Analytical data (^{1}H NMR and MS) are identical to those of enantiomer **7b**.

4.3. *N*-[(*S*)-3-(4-Benzhydryl-piperazin-1-yl)-2-hydroxy-propyl]-4-chloro-*N*-phenethyl benzenesulfonamide (1b)

A mixture of 1-benzhydryl-4-(*S*)-1-oxiranylmethyl-piperazine (**7b**) (1.05 g, 3.41 mmol) and phenethylamine (2.14 mL, 17.04 mmol) in EtOH (50 mL) was heated to reflux overnight. Then solvent was removed under reduced pressure and the crude obtained was purified by flash chromatography (eluent: EtOAc/MeOH 95:5) to afford 0.83 g of (*R*)-1-(4-Benzhydryl-piperazin-1-yl)-3-phenethylamino-propan-2-ol (**8b**) (yield: 57%). To a mixture of **8b** (0.10 g, 0.23 mmol), 4-dimethylaminopyridine (DMAP) (0.003 g, 0.02 mmol) and pyridine (0.04 mL, 0.48 mmol), 4-chlorobenzenesulfonyl chloride (0.05 g, 0.24 mmol) was added. After 16 h stirring at room temperature, 1 N HCl (2 mL) was added, then left stirring for 5 min., and the organic layer was recovered, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting crude was purified by flash chromatography (eluent: EtOAc/MeOH 95:5) to obtain 0.04 g of the title compound (yield: 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.56–2.95 (m, 13H), 3.09 (dd, 1H, J = 14.8, 6.0 Hz), 3.33 (dd, 1H, J = 14.8, 5.6 Hz), 3.30–3.50 (m, 2H), 4.00 (br, 1H), 4.26 (s, 1H), 7.16–7.21 (m, 5H), 7.24–7.30 (m, 6H), 7.39–7.41 (m, 4H), 7.46 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.0 Hz), 7.71 (AA′XX′, 2H, J_{AX} = 8.4 Hz, $J_{AA'XX'}$ = 2.0 Hz). MS: *m*/*z* 604 (M$^+$, 30), 438 (M$^+$-benzhydryl, 100). Chiral HPLC: retention time 28.7 min, 98.6% ee.

4.4. *N*-[(*R*)-3-(4-Benzhydryl-piperazin-1-yl)-2-hydroxy-propyl]-4-chloro-*N*-phenethyl-benzenesulfonamide (1a)

Same procedure as described above starting from 1-benzhydryl-4-(*R*)-1-oxiranylmethyl-piperazine **7a**, through the intermediate (*S*)-1-(4-Benzhydryl-piperazin-1-yl)-3-phenethylamino-propan-2-ol (**8a**), to obtain 0.68 g of the title compound (yield: 51%). Chiral HPLC: retention time 21.3 min, >99% ee. Analytical data (^{1}H-NMR and MS) are identical to those of enantiomer **1b**.

4.5. *N*-[(*S*)-3-(4-Benzhydryl-piperazin-1-yl)-2-hydroxy-propyl]-*N*-benzyl-4-chloro-benzenesulfonamide (2b)

A mixture of 1-benzhydryl-4-(*S*)-1-oxiranylmethyl-piperazine (**7b**) (1.05 g, 3.41 mmol) and benzylamine (1.86 mL, 17.04 mmol) in EtOH (50 mL) was heated to reflux overnight. Then solvent was removed under reduced pressure and the crude obtained was purified by flash chromatography (eluent: EtOAc/MeOH 95:5) to afford 0.64 g of (*R*)-1-(4-Benzhydryl-piperazin-1-yl)-3-benzylamino-propan-2-ol (**9b**) (yield: 45%). To a mixture of **9b** (0.10 g, 0.24 mmol), 4-dimethylaminopyridine (DMAP) (0.003 g, 0.02 mmol) and pyridine (0.04 mL, 0.48 mmol), 4-chlorobenzenesulfonyl chloride (0.05 g, 0.24 mmol) was added. After 16 h stirring at room temperature 1 N HCl (2 mL) was added, then left stirring for 5 min, and the organic layer was recovered, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting crude was purified by flash chromatography (eluent: EtOAc/MeOH 95:5) to obtain 0.01 g (yield: 6%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ

(ppm): 2.03–2.44 (m, 10H), 3.06 (dd, 1H, J = 14.8, 7.2 Hz), 3.27 (dd, 1H, J = 14.8, 4.8 Hz), 3.50–3.60 (m, 1H), 4.18 (s, 1H), 4.33 (d, 1H, J = 14.8 Hz), 4.50 (d, 1H, J = 14.8 Hz), 7.15–7.19 (m, 2H), 7.24–7.28 (m, 9H), 7.37–7.39 (m, 4H), 7.47 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.0 Hz), 7.76 (AA′XX′, 2H, J_{AX} = 8.4 Hz, $J_{AA'XX'}$ = 2.0 Hz). MS: m/z 590 (M^+, 35), 424 (M^+-benzhydryl, 100). Chiral HPLC: retention time 34.4 min, 95% ee.

4.6. *N*-[(*R*)-3-(4-Benzhydryl-piperazin-1-yl)-2-hydroxy-propyl]-*N*-benzyl-4-chloro-benzenesulfonamide (2a)

Same procedure as described above starting from 1-benzhydryl-4-(*R*)-1-oxiranylmethyl-piperazine (**7a**), through the intermediate (*S*)-1-(4-Benzhydryl-piperazin-1-yl)-3-benzylamino-propan-2-ol (**9a**), to obtain 0.02 g of the title compound (yield: 14%). Chiral HPLC: retention time 21.4 min, >99% ee. Analytical data (^{1}H NMR and MS) are identical to those of enantiomer **2b**.

4.7. *N*-[(*S*)-3-(4-Benzhydryl-piperazin-1-yl)-2-hydroxy-propyl]-*N*-phenethyl-benzenesulfonamide (3b)

A mixture of 1-benzhydryl-4-(*S*)-1-oxiranylmethyl-piperazine **7b** (1.05 g, 3.41 mmol) and phenethylamine (2.14 mL, 17.04 mmol) in EtOH (50 mL) was heated to reflux overnight. Then solvent was removed under reduced pressure and the crude obtained was purified by flash chromatography (eluent: EtOAc/MeOH 95:5) to afford 0.83 g of (*R*)-1-(4-Benzhydryl-piperazin-1-yl)-3-phenethylamino-propan-2-ol (**8b**) (yield: 57%). To a mixture of **8b** (0.10 g, 0.23 mmol), 4-dimethylaminopyridine (DMAP) (0.003 g, 0.02 mmol) and pyridine (0.04 mL, 0.48 mmol), benzenesulfonyl chloride (0.03 mL, 0.24 mmol) was added. After 16 h stirring at room temperature 1 N HCl (2 mL) was added, then left stirring for 5 min, and the organic layer was recovered, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting crude was purified by flash chromatography (eluent: EtOAc/MeOH 95:5) to obtain 0.02 g (yield: 16%) of the title compound. ^{1}H NMR (400 MHz, CDCl3) δ (ppm): 2.35–2.41 (m, 8H), 2.61–2.62 (m, 2H), 2.91–2.93 (m, 2H), 3.07 (dd, 1H, J = 14.6, 6.6 Hz), 3.35–3.43 (m, 4H), 3.79 (br s, 1H), 4.21 (s, 1H), 7.15–7.20 (m, 5H), 7.24–7.29 (m, 6H), 7.40–7.41 (m, 4H), 7.47–7.51 (m, 2H), 7.54–7.57 (m, 1H), 7.79–7.81 (m, 2H). MS: m/z 570 (M^+, 30), 404 (M^+-benzhydryl, 100). Chiral HPLC: retention time 28.1 min, 98.7% ee.

4.8. *N*-[(*R*)-3-(4-Benzhydryl-piperazin-1-yl)-2-hydroxy-propyl]-*N*-phenethyl-benzenesulfonamide (3a)

Same procedure as described above starting from 1-benzhydryl-4-(*R*)-1-oxiranylmethyl-piperazine (**7a**), through the intermediate (*S*)-1-(4-benzhydryl-piperazin-1-yl)-3-phenethylamino-propan-2-ol (**8a**), to obtain 0.02 g of the title compound (yield: 13%). Chiral HPLC: retention time 22.4 min, >99% ee. Analytical data (^{1}H NMR and MS) are identical to those of enantiomer **3b**.

4.9. *N*-[(*S*)-3-(4-Benzhydryl-piperazin-1-yl)-2-hydroxy-propyl]-3,4-dichloro-*N*-cyclopropylmethyl-benzenesulfonamide (4b)

A mixture of 1-benzhydryl-4-(*S*)-1-oxiranylmethyl-piperazine (**7b**) (0.90 g, 2.92 mmol) and cyclopropanemethylamine (2.50 mL, 29.22 mmol) in EtOH (70 mL) was heated 6 h to reflux. Then solvent was removed under reduced pressure to afford 1.10 g of (*R*)-1-(4-Benzhydryl-piperazin-1-yl)-3-(cyclopropylmethyl-amino)-propan-2-ol (**10b**) (yield: 97%) without further purification. A mixture of **10b** (0.15 g, 0.40 mmol), 4-dimethylaminopyridine (DMAP) (0.005 g, 0.04 mmol), pyridine (0.06 mL, 0.79 mmol) and 3,4-dichlorobenzene-1-sulfonyl chloride (0.06 g, 0.40 mmol) was shaken overnight at room temperature. Then 1 N HCl (2 mL) was added, the organic layer was recovered, dried over Na_2SO_4, filtered and evaporated under reduced pressure to get a crude that was purified by flash chromatography (eluent: CH_2Cl_2/MeOH 95:5) to obtain 0.12 g (yield: 52%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.02–0.03 (m, 2H), 0.29–0.33 (m, 2H), 0.65 (m, 1H), 2.63 (m, 4H), 2.76–2.78 (m, 2H), 2.95–3.04 (m, 5H), 3.15–3.24 (m, 4H), 4.14 (s, 1H), 4.35 (br s, 1H), 6.97–7.00 (m, 2H), 7.05–7.09 (m, 5H), 7.17–7.19 (m, 3H), 7.39 (m, 2H), 7.66 (m, 1H). MS: m/z 588 (M^+, 35), 422 (M^+-benzhydryl, 100). Chiral HPLC: retention time 13.2 min, 97% ee.

4.10. *N*-[(*R*)-3-(4-Benzhydryl-piperazin-1-yl)-2-hydroxy-propyl]-3,4-dichloro-*N*-cyclopropylmethyl-benzenesulfonamide (4a)

Same procedure as described above starting from 1-benzhydryl-4-(*R*)-1-oxiranylmethyl-piperazine (**7a**), through the interme diate (*S*)-1-(4-Benzhydryl-piperazin-1-yl)-3-(cyclopropylmethyl-amino)-propan-2-ol (**10a**) to obtain 0.09 g of the title compound (yield: 39%). Chiral HPLC: retention time 14.4 min, 98% ee. Analytical data (^{1}H NMR and MS) are identical to those of enantiomer **4b**.

5. Computational methods

The protocol used in this study was the same applied in Ref. 16. Briefly, the target structure has been based on coordinates of the X-ray diffraction BACE1 crystal structure freely available in the RSCB Protein Data Bank[16] (PDB code 1W51[14]). Different docking software have been used: GOLD,[17] FlexX,[18] Glide[19] and Induced Fit Docking.[13] All the inhibitors were built using MOE,[20] energy minimized using MMFF94x force field[21] until a 0.01 energy gradient was attained, and atomic charges assigned using AM1-BCC semi-empirical partials charge.[22] The top scored final docking poses for all of the inhibitors were compared with the pharmacophore proposed by Limongelli et al.[23] created using the MOE computational suite.

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References and notes

1. Glenner, G. G.; Wong, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 885.
2. Ballatore, C.; Lee, V. M.-Y.; Trojanowski, J. Q. *Nat. Rev. Neurosci.* **2007**, *8*, 663.
3. Forman, M. S.; Trojanowski, J. Q.; Lee, V. M.-Y. *Nat. Med.* **2004**, *10*, 1055.
4. Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappenman, K. E.; Kawabe, T.; Kola, I.; Kuehn, R.; Lee, M.; Liu, W.; Motter, R.; Nichols, N. F.; Power, M.; Robertson, D. W.; Schenk, D.; Schoor, M.; Shopp, G. M.; Shuck, M. E.; Sihna, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. *Hum. Mol. Genet.* **2001**, *10*, 1317.
5. John, V.; Beck, J. P.; Bienkowski, M. J.; Sinha, S.; Heinrikson, R. L. *J. Med. Chem.* **2003**, *46*, 4625.
6. Some recent examples are reported in: (a) Barrow, J. C.; Rittle, K. E.; Ngo, P. L.; Selnick, H. G.; Graham, S. L.; Pitzenberger, S. M.; McGaughey, G. B.; Colussi, D.; Lai, M.-T.; Huang, Q.; Tugusheva, K.; Espeseth, A. S.; Simon, A. J.; Munshi, S. K.; Vacca, J. P. *ChemMedChem* **2007**, *2*, 995; (b) Maillard, M. C.; Hom, R. K.; Benson, T. E.; Moon, J. B.; Mamo, S.; Bienkowski, M.; Tomasselli, A. G.; Woods, D. D.; Prince, D. B.; Paddock, D. J.; Emmons, T. L.; Tucker, J. A.; Dappen, M. S.; Brogley, L.; Thorsett, E. D.; Jewett, N.; Sinha, S.; John, V. *J. Med. Chem.* **2007**, *50*, 776.

7. BACE-inhibition assays have been carried out as reported in: Magnoni, L.; Terstappen, G. C.; Fecke, W. *Assay Drug Dev. Technol.* **2005**, *3*, 287.
8. QikProp, version 3.0, Schrödinger, LLC, New York, NY, 2007.
9. Luco, J. M. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 396.
10. Kelder, J.; Grootenhuis, P. D.; Bayada, D. M.; Delbressine, L. P.; Ploemen, J. P. *Pharm. Res.* **1999**, *16*, 1514.
11. Ajay; Bemis, G. W.; Murkco, M. A. *J. Med. Chem.* **1999**, *42*, 4942.
12. Fujimoto, T.; Matsushita, Y.; Gouda, H.; Yamaotsu, N.; Girono, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2771.
13. Schrödinger Suite 2007 Induced Fit Docking protocol; Glide version 4.5, Schrödinger, LLC, New York, NY, 2005; Prime version 1.6, Schrödinger, LLC, New York, NY, 2005.
14. Patel, S.; Vuillard, L.; Cleasby, A.; Murray, C. W.; Yon, J. *J. Mol. Biol.* **2004**, *343*, 407.
15. Asso, V.; Ghilardi, E.; Bertini, S.; Digiacomo, M.; Granchi, C.; Minutolo, F.; Rapposelli, S.; Bortolato, A.; Moro, S.; Macchia, M. *ChemMedChem* **2008**, *3*, 1530.
16. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res* **2000**, *28*, 235.
17. Hartshorn, M. J.; Verdonk, M. L.; Chessari, G.; Brewerton, S. C.; Mooij, W. T. M.; Mortenson, P. N.; Murray, C. W. *J. Med. Chem.* **2007**, *50*, 726.
18. Stahl, M. *Perspect. Drug Discovery Des.* **2000**, *20*, 83.
19. Glide, version 4.5, Schrödinger, LLC, New York, NY, 2007.
20. MOE (The Molecular Operating Environment) Version 2006.08, software available from Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, Canada H3A 2R7. http://www.chemcomp.com.
21. Halgren, T. *J. Comput. Chem.* **1996**, *17*, 490.
22. Jakalian, A.; Jack, D. B.; Bayly, C. I. *J. Comput. Chem.* **2002**, *23*, 1623.
23. Limongelli, V.; Marinelli, L.; Cosconati, S.; Braun, H. A.; Schmidt, B.; Novellino, E. *ChemMedChem* **2007**, *2*, 667.