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α-Naphthylaminopropan-2-ol Derivatives as BACE1 Inhibitors

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Alzheimer's disease (AD) causes progressive neurodegeneration; brain neurons of people affected by this pathology are characterized by the presence of extracellular senile plaques, mainly consisting of amyloid β (Aβ) peptide aggregates,[1] and intracellular neurofibrillary tangles caused by the aggregation of tau proteins,[2] both of which are proposed to play a key role in the progression of AD.[3]

The formation of the Aβ peptide from the amyloid precursor protein (APP) is catalyzed by BACE1 (β-secretase), a proteolytic enzyme belonging to the aspartyl protease family, widely recognized as a potential therapeutic target for the treatment of AD.[4] Many known BACE1 inhibitors incorporate a hydroxyethylamine (HEA) transition state isostere moiety.[5] Some of the most potent inhibitors are peptidomimetic structures or possess relatively high molecular weights, however, BACE1 is found inside the central nervous system (CNS) and, therefore, blood–brain barrier (BBB) permeation is an additional issue to be addressed, for example, by a reduction in molecular weight and in the overall polarity of the drug candidate.

To identify new BACE1 inhibitors, a series of commercially available HEA-derived molecules was screened using a recently developed high-throughput screening (HTS) fluorescence assay.[6] Compound **1**, a dibromo-substituted carbazole containing an α-naphthylaminopropan-2-ol portion, was identified by the HTS assay as a good inhibitor of BACE1 with an IC_{50} value of 1.1 μM. A series of compound **1** analogues was designed and synthesized, keeping the aminoalcohol moiety intact and varying the heterocyclic terminal group (Figure 1).

The choice of the heterocyclic moiety was directed by three factors; i) reduction of the molecular weight of **1**, ii) structural resemblance to **1**, and iii) availability of synthetic precursors. The carbazole- and indole-derived heterocycles were selected to replace the 3,6-dibromocarbazole moiety in compound **1.** The closely related carbazole derivatives synthesized include unsubstituted carbazole (**2a**), 3,6-dichlorocarbazole (**2b**), and 1,2,3,4-tetrahydrocarbazole (**2c**), as well as the substituted indoles (**2d**–**f**) (Table 1).

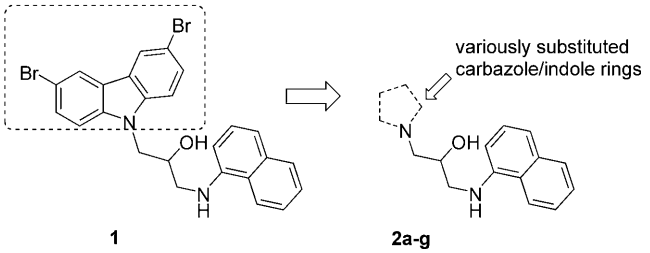

Figure 1. The design of a series of α-naphthylaminopropan-2-ol derivatives **2a**–**g** inspired by the commercially available BACE1 inhibitor **1**.

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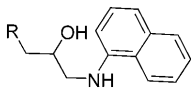
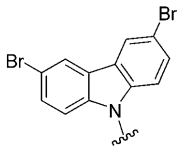
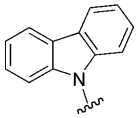
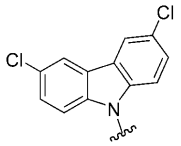
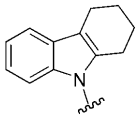
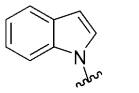
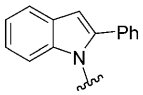
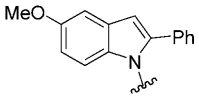
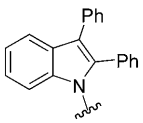
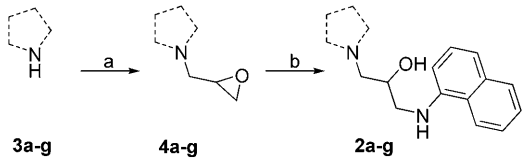
Table 1. Structures, MW, BACE1 inhibitory activities and predicted BBB permeation of compounds **2a**–**g**, together with HTS hit **1**.



Compound	R	MW [Da]	BACE1 IC_{50}[a] [μM]	$LogBB_{pred}$[b]
1		524	1.10	0.2
2a		366	2.99	−0.2
2b		435	0.50	0.2
2c		370	>10	−0.2
2d		316	>10	−0.2
2e		392	1.66	−0.2
2f		423	>10	−0.3
2g		469	>10	−0.2

[a] IC_{50} measurements were performed as described in [6]. 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 mean. [b] Predicted BBB permeation (LogBB = Log [brain]/[blood])[9]

Compounds **2a-g** were obtained by a straightforward synthesis starting from commercially available heterocyclic precursors (**3a–g**). Treatment of the appropriate heterocycle **3a–g** with KOH in anhydrous DMF at −10 °C, followed by the slow addition of a solution of epichlorohydrin in DMF, afforded epoxides **4a–g** in moderate to good yields (54–90%). Simple aminolysis of these epoxides in ethanol proceeded poorly, with low conversion and poor regioselectivity, and was not improved by heating the reaction. The addition of sub-stoichiometric amounts of Lewis acids was explored in an attempt to improve the epoxide ring opening step. The use of 0.1 equivalents of bismuth(III) chloride[7] promoted an efficient and fully regioselective ring opening aminolysis of epoxides **4a–g** with α-naphthylamine in cyclohexane, to give the desired aminoalcohols **2a–g** in good yields (50–70%) (Scheme 1).



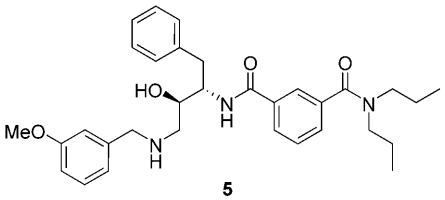
Scheme 1. Reagents: a) epichlorohydrin (2.5 equiv), KOH (1.2 equiv), anhyd DMF, −10 to 0 °C , 4–6 h, 54–90%; b) α-naphthylamine (2.0 equiv), $BiCl_3$ (0.1 equiv), *c*-C_6H_{12}, RT, 2–4 h, 30–60%, 2 steps.

BACE1 inhibitory activities of compounds **2a–g** were determined using a fluorescence-based assay,[6] and the results are shown in Table 1. While all the newly synthesized compounds have molecular weights lower than HTS hit **1**, presumably leading to better BBB permeation, other properties are known to be important for BBB permeation, such as the polar surface area, structural rigidity, lipophilicity and the hydrogen bond donor capacity of a molecule.[8] For this reason, in silico evaluation of compounds **2a–g** was used to predict the LogBB, defined as the ratio of the steady-state concentrations of a compound in the brain to those in the blood (LogBB = log [brain]/[blood]). Compound **2b** was predicted to have the highest LogBB, theoretically capable of crossing the BBB and reaching a concentration in the CNS 1.5 times higher than in the blood stream.

Among the carbazole derivatives, compound **2a**, the non-halogenated analogue, exhibits a threefold reduction in the inhibitory ability compared to the lead compound **1** (IC_{50} = 2.99 μM vs. IC_{50} = 1.10 μM). On the other hand, replacement of the bromo substituents of compound **1** with chlorine atoms (compound **2b**), leads to a twofold increase in BACE1 inhibition (IC_{50} = 0.50 μM). A partial reduction of the carbazole system gives rise to compound **2c**, with exhibits negligible inhibitory activity ($IC_{50} \geq 10$ μM). Of the indole series, only the 2-phenylindolyl derivative **2e** showed an appreciable IC_{50} value (IC_{50} = 1.66 μM), whereas the other indoles (**2d**, **2f**, and **2g**) displayed IC_{50} values greater than 10 μM.

These results show that when the molecular size of the tricyclic portion of **1** is reduced by removing the 3,6-dibromo substituents, the BACE1 inhibition is still preserved, although to a decreased level (compound **2a**, ΔMW = −158 Da). The inhibitory potency improved when a 3,6-dichloro-substitution pattern is introduced (compound **2b**), although in this case the decrease in molecular weight is less pronounced (ΔMW = −89 Da). Both the partial reduction and loss of π character in one of the aromatic rings of the carbazole system (compound **2c**), and the contraction of the carbazole to an unsubstituted indole (compound **2d**) causes a loss of BACE1 inhibitory activity, whereas the reintroduction of a third aromatic ring, as in 2-phenylindole derivative **2e**, restores an appreciable activity in the low micromolar range, accompanied by a moderate reduction in molecular weight when compared to compound **1** (ΔMW = −132 Da). Taken together, these observations confirm the importance of the system comprised of three aromatic rings, either all condensed as in completely aromatic carbazoles **2a–b**, or partially condensed as in 2-phenylindole derivative **2e**. Both the introduction of an electron-donating group at the 5 position (compound **2f**) or an additional phenyl substituent at the 3 position (compound **2g**) resulted in a loss of activity, probably by disrupting π interactions within the active site. Furthermore, the remarkable inhibition potency preserved after a considerable reduction in molecular weight (compounds **2a** and **2e** cf. **1**) suggests further biological evaluation, such as cellular assays, would also be promising.

Molecular docking studies were conducted in an attempt to understand the interactions of compounds **2a–g** with BACE1. The flap region of BACE1 can adopt open and closed conformations in dynamic equilibrium, as demonstrated by several crystal structures.[10] The flexibility of this flap region further complicates the conformational space within the active site and consequently makes BACE1 a challenging therapeutic target. Interestingly, a detailed analysis of the available 3D data highlights how this particular region can undergo a considerable rearrangement during ligand binding in a way that generally seems to favor a closed conformation.[10] For this reason, a flap-closed conformation (PDB code 1W51[10]), containing a co-crystallized potent inhibitor belonging to the HEA structural class (compound **5**), was used in the molecular modeling studies. Both enantiomers of the inhibitors **2a–g** were docked in the BACE1 binding pocket using three different docking programs. The aim was to define a binding mode hypothesis able to justify the observed structure–activity relationships identified.



The use of different search algorithms during the posing step of the docking study exhaustively explored the conformational space occupied by the ligand in the binding site. All probable binding modes were compared with the pharmacophore model proposed by Limongelli and co-workers.[11] This

pharmacophore represents a collection of the essential physicochemical features crucial for ligand recognition and BACE1 inhibition, and was derived from six different inhibitors in their biologically active conformations.[9] In particular, conformational structures identified in the post-processing docking step that matched the proposed pharmacophore features were clustered. In fact, the newly synthesized compounds are characterized by strict geometrical and steric properties. First of all, the distance between the two bulky ring systems at either end of the molecule is insufficient to allow the hydroxyethyl moiety to adopt the same orientation found in other known BACE1 inhibitors. Consequently, this scaffold cannot form hydrogen bonds with both the catalytic aspartic acids of BACE1 (Asp 32 and Asp 228). This peculiarity is even more interesting, considering that analogue **2b** exhibits sub-micromolar activity regardless.

Interestingly, the available X-ray diffraction information shows that flap-closed BACE structures are extremely similar, even with different inhibitors bound to the active site. However, there are a few differences arising from the flexibility of some amino acid residues. Based on this consideration, and on the intrinsic limitation of docking programs to accurately simulate ligand poses in a flexible binding site, we decided to use the Induced Fit[12] docking protocol to include the reorganization of the side chains of the amino acids within the binding site, and to allow slight backbone movements through energy minimization.[13] The final binding mode predicted for compound **2b** suggests an induced fit effect of the ligand on the side chain orientations of the active site residues, in particular, Gln 73 and Arg 128 (Figure 2 A), which are part of the most flexible residues in the flap-closed conformation, verified by superimposition of the X-ray structures. In this docking pose, the strongest polar interaction is with only one of the two catalytic aspartic acids (Asp 228). This key interaction orients the naphthalene ring toward S2′ pocket, where it can interact through van der Waals interactions with Tyr 198, Val 69 and Ile 126. On the opposite side, the orientation of the chloro-carbazole moiety allows hydrophobic interactions with both S1 and S2 pockets (Figure 3). Following this binding mode hypothesis, the observed structure–activity relationships can be rationalized by comparing the bulkiness of the substituents interacting with both S1 and S2 pockets. Bulk substituents are not accommodated in these pockets, and result in the disruption of the key hydrogen bond between hydroxyethyl moiety and Asp 228, such as derivatives **2c**, **2f** and **2g**. Moreover, the presence of smaller substituents result in inactive compounds since they lose the important hydrophobic interactions with the S2 pocket, as shown for compound **2d**. Interestingly both derivatives **2a** and **2e**, the least and most bulky compounds of the series, respectively, are both active, and are able to bind to the BACE1 binding cleft in a slightly different arrangement. In fact, the "Induced Fit" docking results for derivative **2a** propose a binding mode in which the molecule is rotated by 180° where the carbazole moiety interacts with S2′ pocket, while the naphthyl group is placed in S1, in comparison to derivative **2b**. Conversely, compound **2e** shows a hydrophobic based binding mode interacting principally with the S1 and S2 pockets, and only weakly with Asp 228.

Another possible advantage of using this scaffold over others shown by the docking studies is that the particular position of the hydroxy group allows an interaction with the aspartic acid in both its enantiomeric forms (Figure 2 A). In this case, the racemic mixture should show a similar activity to the two pure enantiomers, and the complex chiral resolution process would not be necessary.[14] Similar evidence has been reported by Fujimoto and co-workers in their recently described application based on an in silico multi-filter approach,[15] further confirming a minor role of the chirality of the alcohol center present in the HEA pharmacophore, at least in the preliminary stages of BACE1 inhibitor discovery.

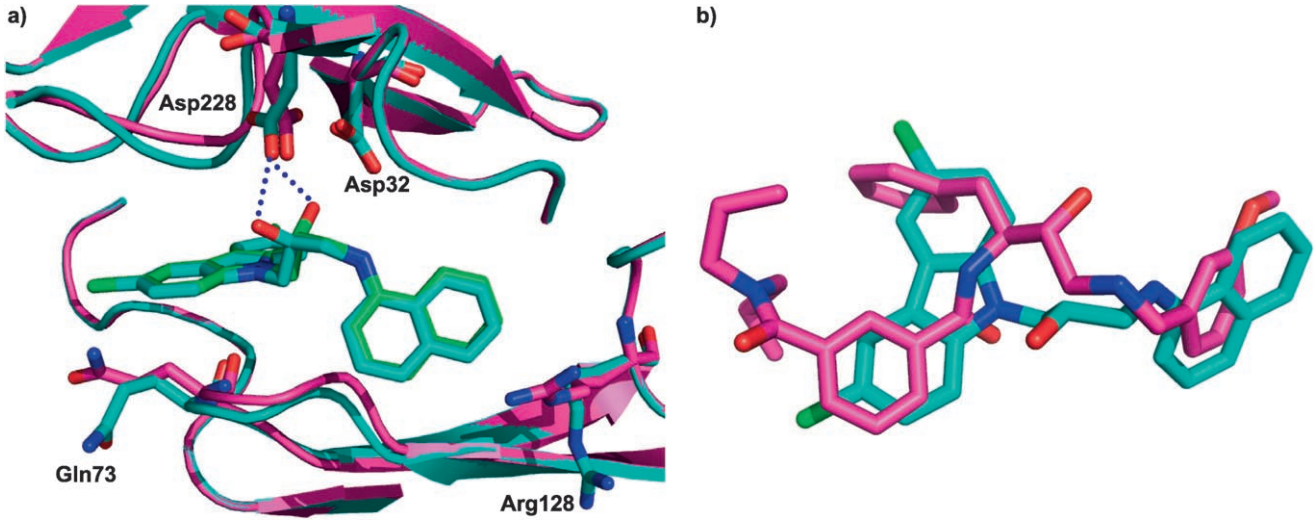


Figure 2. The binding mode of compound **2b** in the crystal structure (PDB 1W51[10]) resulting from the Induce Fit Docking protocol:[12] a) Comparison of the BACE1 active site conformation in the unbound state (magenta) and after interaction with inhibitors (*S*)-**2b** (cyan) and (*R*)-**2b**; b) Superimposition of compound **5** from the crystal structure (magenta) and the docking pose of inhibitor (*S*)-**2b** (cyan).

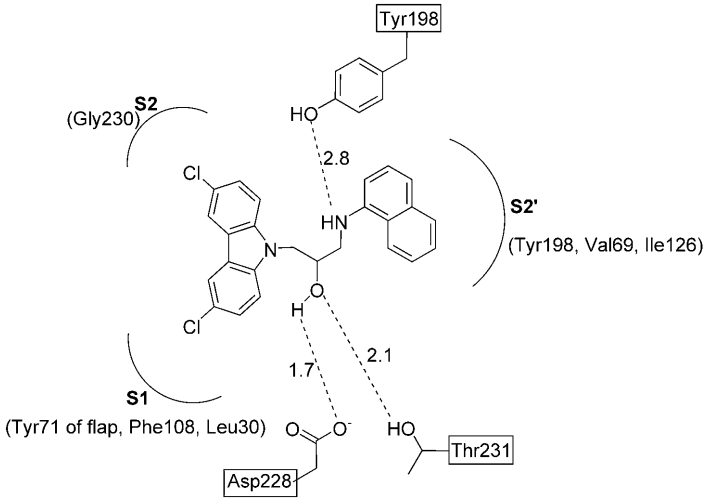


Figure 3. Schematic representation of the principal interactions between inhibitor **2b** and the BACE1 active site indicated by the docking studies. Hydrogen bonds are shown as dotted lines, important distances are given in Å, and the principal amino acids involved in van der Waals interactions are shown in parentheses.

Compound **2b** represents a new molecule potentially able to cross the BBB, and to inhibit BACE1 with an unusual binding mode, sharing some common features with other hydroxyethylamino-derived inhibitors, but at the same time with remarkable differences (Figure 2B). In particular, the fact that the hydroxy moiety of this scaffold does not interact with Asp 32, but is involved in a key interaction with Asp 228 irrespective of the chirality, suggests compound **2b**, as well as the simpler analogues **2a** and **2e**, are promising lead compounds for further optimization to improve BACE1 inhibition. In particular, the superimposition of **2b** on compound **5**, (Figure 2B) indicates how it could be possible to create derivatives able to interact with the S3 pocket of the enzyme (adjacent to S1, not defined in Figure 3), occupied by the terminal amide group of compound **5**.[10] Indeed, further substitution of the carbazole group may be designed to mimic the role of the di-*n*-propylamide moiety of compound **5**. Not only could these additional interactions further improve the binding free energy, but they could also address possible selectivity issues. In fact, this site is believed to be crucial to achieve a selective action on BACE1, when compared to other aspartyl proteases.

Experimental Section

Synthesis of compound 2b: A solution of 3,6-dichlorocarbazole (5.00 g, 21.2 mmol) in anhyd DMF (70 mL) was treated with KOH (1.43 g, 25.4 mmol) at RT and the resulting mixture was stirred for 1 h at RT. The reaction was cooled to $-10\,^{\circ}C$ and treated dropwise with a solution of epichlorohydrin (4.9 g, 53 mmol) in anhyd DMF (20 mL) then stirred for 6 h at $0\,^{\circ}C$. The reaction was poured onto ice and extracted with EtOAc (2×250 mL). The combined organic phase was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography (*n*-Hexane, 100%) gave epoxide **4b** as a yellow oil (3.09 g, 50% yield): ^1H NMR (200 MHz, $CDCl_3$): $\delta=2.52$ (dd, $J=4.8$, 2.6 Hz, 1H), 2.81 (*pseudo*-t, $J=4.3$ Hz, 1H), 3.32–3.39 (m, 1H), 4.40 (dd, $J=15.8$, 4.7 Hz, 1H), 4.63 (dd, $J=15.8$, 3.3 Hz, 1H), 7.22–7.30 (m, 2H), 7.46–7.49 ppm (m, 4H). A 2 M solution of this epoxide (1.0 g, 3.4 mmol) in *c*-C_6H_{12} was treated with α-naphthylamine (1.0 g, 7.0 mmol) and $BiCl_3$ (0.11 g, 0.34 mmol) at RT and Stirred for 4 h. The reaction was diluted with a saturated aq $NaHCO_3$ (50 mL) and extracted with EtOAc ($3\times$ 50 mL). The combined organic phase was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography (*n*-Hexane/EtOAc, 9:1) gave the aminoalcohol **2b** as a light yellow oil (0.84 g, 57% yield): ^1H NMR (200 MHz, $CDCl_3$): $\delta=3.28$ (dd, $J=$ 12.7, 6.8 Hz, 1H), 3.43–3.51 (m, 2H), 4.28–4.44 (m, 2H), 7.08–7.56 (m, 11H), 7.75–7.90 ppm (m, 2H).

BACE1 inhibition fluorescence assays: The fluorescence assays were conducted as previously reported,[6] and the concentration-response curves for compounds **1** and **2a-g**, with human recombinant BACE1 (Invitrogen, Carlsbad, CA) and BACE1 TR-FRET substrate [acetyl-C(W8044-Eu)-EVNLDAEFK-QSY7] (Perkin–Elmer Life Sciences, Turku, Finland) were determined. Briefly, enzymatic reactions were performed in 50 mM NaOAc and 0.005% Triton X-100 (pH 4.5), in a final volume of 30 µL with 200 nM of substrate and 6 nM of enzyme. Inhibitors were tested in solutions containing 3.3% of DMSO. Time resolved fluorescence was measured using the Analyst® GT plate reader (Molecular Devices, Sunnyvale, CA) with a filter set allowing measurements at an excitation wavelength of 330 nm and an emission wavelength of 615 nm.

Computational methods: X-ray diffraction BACE1 crystal structure coordinates in complex with the inhibitor compound **5** were downloaded from the RSCB Protein Data Bank[16] (PDB code 1W51[10]). Docking studies were performed using default options with GOLD,[17] FlexX[18] implemented in the Operating Environment (MOE 2006.08),[19] Glide,[20] and Induced Fit Docking.[12] In the first two programs, the protein and ligands were prepared using MOE.[19] Waters molecules and the inhibitor were removed from the BACE1 crystal structure, hydrogen atoms were added, and the energy minimized using the AMBER99 force field[21] until the energy gradient reached 0.05 kcal mol^{-1}. Both enantiomers of all the inhibitors were built using MOE,[19] energy minimized using MMFF94x force field[22] until a 0.01 energy gradient was attained, and atomic charges assigned using AM1-BCC semi-empirical partials charge.[23] For Glide[20] and Induced Fit Docking,[12] the protein preparation module of Maestro[24] and LigPrep[25] were used for macromolecule and inhibitors optimization, respectively. Hydrogen atoms were added to the heavy atoms in the protein; possible conflictions in the hydrogen bonding were corrected, and the two alternate tautomers of the histidine side chain were considered, especially if near the binding pocket. Using the OPLS force field, the hydrogenated BACE1–inhibitor **5** complex was energy minimized until the average RMS deviation of the non-hydrogen atoms reached 0.3 Å. Final docking poses for all of the inhibitors were compared with the pharmacophore proposed by Limongelli et al.[11] created using the MOE[19] computational suite.

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Keywords: Alzheimer's disease · amino alcohols · BACE1 · molecular modeling · inhibitors

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