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Synthesis and structure–activity relationship studies of 1,3-disubstituted 2-propanols as BACE-1 inhibitors

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ABSTRACT

A library of 1,3-disubstituted 2-propanols was synthesized and evaluated as low molecular weight probes for β-secretase inhibition. By screening a library of 121 1,3-disubstituted 2-propanol derivatives, we identified few compounds inhibiting the enzyme at low micromolar concentrations. The initial hits were optimized to yield a potent BACE-1 inhibitor exhibiting an IC_{50} constant in the nanomolar range. Exploration of the pharmacological properties revealed that these small molecular inhibitors possessed a high selectivity over cathepsin D and desirable physicochemical properties beneficial to cross the blood–brain barrier.

The protease BACE-1 (also known as β-secretase) is a validated target for the development of inhibitory compounds with potential as a treatment for Alzheimer's disease (AD).[1–3] BACE-1 is an aspartic protease which cleaves the amyloid precursor protein (APP), a transmembrane protein, into a 42 amino acid peptide also known as the amyloid β (Aβ).[4–6] Though, the exact mechanisms of events leading to AD are still under scrutiny, recent reports suggest that the insoluble Aβ peptide is neurotoxic causing the neurodegeneration typically observed in AD.[6,7] Furthermore, it has been shown that BACE-1 knockout mice not only produce Aβ at significantly reduced amounts, but also do not develop the typical pathology of AD.[8–10] The inhibition of BACE-1, therefore, is considered to be among the best strategies to reduce the undesired Aβ production and to treat AD.

In general, the development of efficacious BACE-1 inhibitors is difficult due to the requirement that the inhibitory compounds must not only be potent, but also display selectivity over other aspartic proteases and have good physicochemical properties to penetrate the blood–brain barrier.[11] Furthermore, the elongated BACE-1 active site dimensions challenge medicinal chemists to design a small molecule that efficiently occupies the active site.[12,13] Peptidomimetics approach with transition state isosteres has been one of the successful strategy for developing BACE-1 inhibitors.[1–3] For example, hydroxyethylamines (HEAs) are known to broadly mimic the transition state of aspartyl proteases' substrates[14–16] and they have been successfully incorporated in BACE-1 inhibitory compounds.[17–23] Macchia and co-workers previously reported the study of a small library of eight hydroxyethylamine-containing BACE-1 inhibitors exhibiting IC_{50} values in the low μM range.[19,20] Extensive docking studies with compound **1** suggests that the hydroxyl group tightly interacts with the aspartic acid residue Asp 228 positioning the carbazole moiety in the S1 and S2 subpockets, while the 1-naphthylamine interacts in the S2′ pocket via a

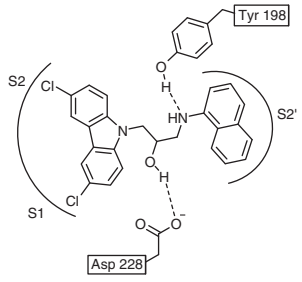

Figure 1. Schematic representation of the main interactions between BACE-1 inhibitor **1** and the BACE-1 active site. Hydrogen bonds are shown as dotted lines.[19]

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; APP, amyloid precursor protein; HEA, hydroxyethylamines; BACE-1, β-secretase.

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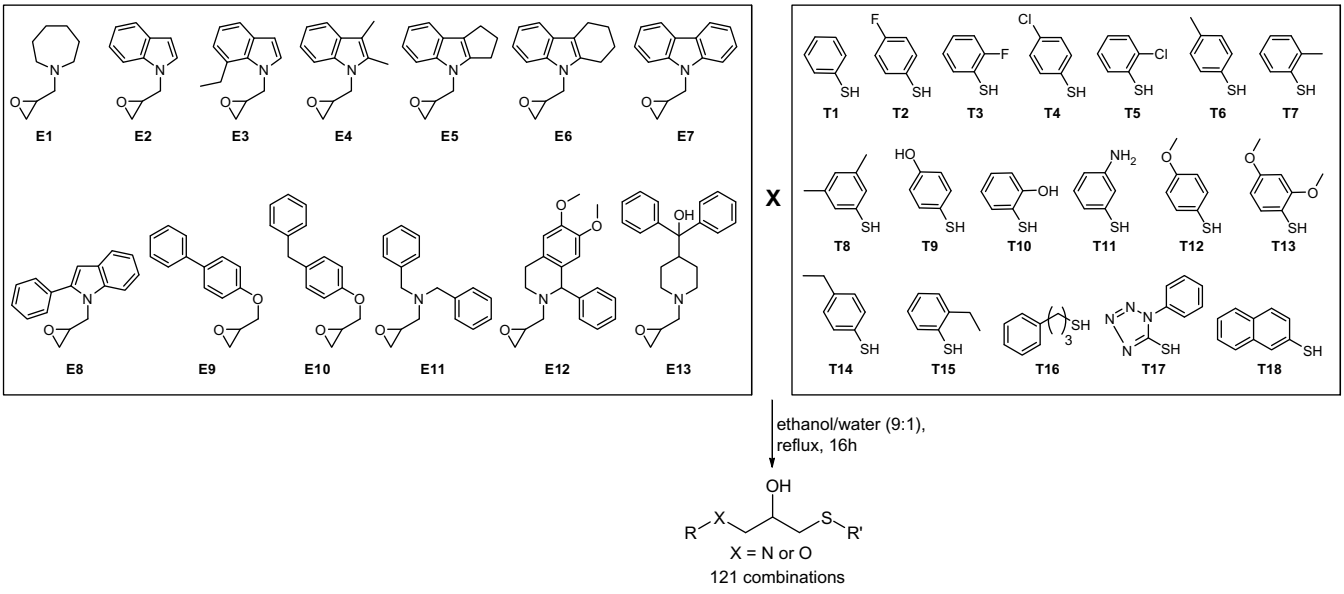
hydrogen bond between the nitrogen and tyrosine 198 and Van der Waals interactions with tyrosine Tyr 198, valine Val 69, and isoleucine Ile 126 (Fig. 1).[19]

As these 1,3-disubstituted 2-propanol derivatives possess low μM potency, small molecule size and attractive synthetic tractability we decided to conduct an extensive investigation on this molecular scaffold for BACE-1 inhibition. A diverse set of indoles, secondary amines and carbazoles derivatized with an epoxide group was synthesized and further reacted with commercially available thiols to yield a library of 1,3-disubstituted 2-propanols (Fig. 2A). All reactions were performed at 50–100 mg scale and the products were purified by column chromatography and subsequently characterized by ^{1}H NMR, ^{13}C NMR, and MS. Besides the previously reported carbazole and indole containing scaffolds, the library design was further expanded by a biphenyl (**E9**), a diphenylmethane (**E10**) and various secondary amines (**E1**, **E11**, **E12** and **E13**). Although, not all possible compound combinations were prepared and tested, a selection of 121 1,3-disubstituted 2-propanols was synthesized and tested to provide reliable information about epoxide thiol combinations that yield promising BACE-1 inhibitors. The whole 1,3-disubstituted 2-propanol library of compounds was initially tested at 100 μM concentration using a detergent-containing fluorescence resonance energy transfer (FRET) based BACE-1 assay, which previously has been successfully used in identifying BACE-1 inhibitors.[24–26] Of these 121 compounds, the compounds that showed activity at 100 μM were then screened at both 50 and 25 μM compound concentration. Although this simple screening approach did not provide very precise inhibition data, it provided valuable structure–activity trends important for hit optimization. The majority of the compounds that displayed activity contain at least one extended ring system such as a carbazole, a tetrahydro-carbazole, or a 2-phenyl-indole, while

A



B

Compound		Epoxides E1			E2			E3			E4			E5			E6			E7			E8			E9			E10			E11			E12			E13		
Concentration (μM)		100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25
Thiol	T1	2									69			85	64	35				86	85	79											0						2		
	T2	10			54			86	41	48							87	92	56										62			2			15					
	T3										56			94	82	51	95	86	68	89	84	67								64			1			23			8		
	T4	0																					82	69	56				63			24			3			10		
	T5							99	87	65	100	93	68				92	99	68										81	73	62	45						5		
	T6													91	81	55							76	65	45	27						7			11			1		
	T7													92	89	73							67			74									24			5		
	T8	15						90	71	40																			71			38			15			0		
	T9										75												86	77	72	17			94	100	85							0		
	T10				24			32			61												86	102	87	33			84	101	90	2						3		
	T11										55									100	87	69	73																	
	T12							83	54	11	96	70	26	85	74	27	87	90	58	88	72	37	70			45			48			10			20			0		
	T13				84	76	43	69			83	37	0	75			74			80	69	22	77	42	21	74			67			34			24			0		
	T14				72			92	61	38													79	78	42				69						21			1		
	T15																						80	72	60				71			54			1			3		
	T16							0						96	81	53																16								
	T17	0			58			86	46		93	64	31	87	76	37				64			53			71			62			0			45			1		
	T18	0									87			87	80	43				76	74	64										19						0		

Legend: Percent inhibition is color coded

not tested	< 60 %	60%	70%	80%	90%

Figure 2. Synthesis and screening of a library of 1,3-disubstituted-2-propanols. (A) Preparation of the initial library starting from epoxides and commercially available thiols. Of the 234 possible compounds, 121 analogues have been synthesized. (B) A set of 121 compounds have been tested initially at 100 μM concentration. Compounds displaying 75% inhibition or greater have been further tested for BACE-1 inhibition at 50 and 25 μM concentrations. The inhibition data for the screening at 100, 50, and 25 μM compound concentrations have been given as percent inhibition. Percent inhibition data is the average of two or more independent measurements.

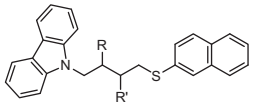
Table 1
IC_{50} values of selected compounds identified in the screening

Compound	BACE-1 IC_{50} (μM)	Cathepsin D IC_{50} (μM)
E2T18	10.5 ± 1.0	n.d.
E3T5	7.52 ± 1.13	>300
E5T7	21.4 ± 1.5	n.d.
E6T3	11.0 ± 1.4	n.d.
E6T5	8.28 ± 0.78	>300
E6T12	15.2 ± 1.4	n.d.
E7T3	5.36 ± 0.38	>300
E7T11	36.1 ± 7.8	n.d.
E7T18	4.93 ± 0.86	>300
E8T9	13.6 ± 1.3	n.d.
E8T10	25.4 ± 6.1	n.d.
E8T15	10.3 ± 0.8	n.d.
E8T18	6.66 ± 1.01	>300
E10T5	9.60 ± 1.20	>300
E10T9	10.7 ± 2.0	n.d.

compounds with an indole ring or small aromatic residues are less potent. Furthermore, compounds containing basic amine were completely inactive, though these comprise aromatic residues.

All compounds that showed an activity of 50% inhibition or greater at 25 μM were then tested at multiple concentrations to establish an inhibitory concentration IC_{50}. The inhibitory concentrations were determined for 16 compounds resulting in a range of IC_{50} constants from 4.93 to 36.1 μM. The best inhibitor was found to be **E7T18** with an IC_{50} of 4.93 μM (Table 1), while 2-phenyl-indole **E8T18** was slightly less potent. One of the most prominent bottlenecks in identifying lead molecules for BACE-1 inhibition is developing inhibitors with selectivity for BACE-1 over other essential aspartyl proteases. Compounds displaying IC_{50}s in the single digit μM range were further assessed for their selectivity to inhibit BACE-1 over cathepsin D. Cathepsin D is an aspartyl protease with a catalytic domain similar to BACE-1 and is ubiquitously present in almost all cells.[12,27] Importantly, none of the tested compounds showed any significant inhibition of cathepsin D up to 300 μM compound concentration rendering the best compounds, **E7T18** and **E8T18**, a selectivity of at least 50 fold. As reported by Shoichet and co-workers, lead identification can be compromised by promiscuous compounds that act as noncompetitive inhibitors by aggregate formation leading to false positives.[28,29] As put forth by Seidler and co-workers, among the important criteria in assessing aggregation-based promiscuity include non-specific activity and high sensitivity to the presence of detergents.[29] The fact that the lead inhibitors show a good selectivity for BACE-1 over cathepsin D and the presence of 0.05% detergent in the assay buffer to disrupt aggregates are good indicators that the lead inhibitors are likely to be valid hits for BACE-1.

Table 3
IC_{50} values of **E7T18** analogues with various C4-linkers

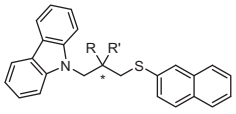


Compound	R	R′	BACE-1 IC_{50} (μM)	Cathepsin D IC_{50} (μM)
9	H	OH	4.31 ± 0.66	>300
10	OH	H	6.74 ± 0.50	>300
11	OH	OH	10.1 ± 1.0	n.d.

Next, studies have been undertaken to investigate the linker connecting the carbazole or 2-phenyl-indole to the naphthyl ring. This linker optimization focused on compound **E7T18**, since it is the most potent compound. Initially, analogues have been synthesized to evaluate whether the alcohol group is important for activity. Complete removal of the hydroxyl group in compound **2** is combined with a decrease in activity (Table 2). Next, the enantiomers of **E7T18** were synthesized independently and tested against BACE-1. The *S* enantiomer **3** with an IC_{50} of 5.39 μM seems to bind to BACE-1 with an affinity similar to the racemic compound **E7T18**, whereas the *R* enantiomer **4** was found to be slightly less active. Importantly considering the standard deviations to be in the range of 0.8–1.1 μM, the small differences between the IC_{50}s of compounds **E7T18**, **3** and **4** support the previous docking studies by Macchia and co-workers, in which the hydroxyl group of HEA inhibitor interacts with Asp 238 independently of the alcohol's stereochemistry.[19] With the idea to improve the interaction between the compound's linker and BACE-1, other groups were introduced as replacements of the alcohol. The potency dramatically dropped for ketone **5** possibly due to an unfavorable interaction between the negatively charged Asp 238 and the ketone's dipole moment. Similarly, primary amines **6** and **7**, as well as piperazine derivative **8** did not improve the activity against BACE-1.

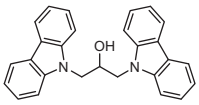
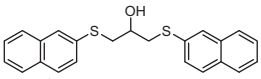
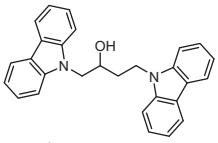
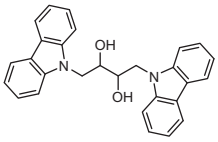
Next, a small set of compounds was designed with the aim to understand whether a compound with the linker length of 4 methylenes and an alcohol group would improve the potency in comparison to **E7T18**. Mono-alcohols **9** and **10**, and diol **11** were synthesized and tested for BACE-1 inhibition. Diol **11** showed decreased inhibition, while mono-alcohols **9** and **10** possessed single digit μM IC_{50} values. Compound **9**, whose alcohol functionality is positioned on the linker closer to the napthyl ring compared to the carbazole moiety, demonstrated the IC_{50} value of 4.31 μM and thus was slightly better than the initial hit **E7T18**. These studies on the linker (Tables 2 and 3) reveal that the linker has to be sterically compact and can accommodate only one hydroxyl group.

Table 2
IC_{50} values of **E7T18** analogues modified at the linker



Compound	R and R′	BACE-1 IC_{50} (μM)	Cathepsin D IC_{50} (μM)
2	R = R′ = H	7.52 ± 1.13	>300
3	R = OH, R′ = H, S-configuration	5.39 ± 0.81	>300
4	R = H, R′ = OH, R-configuration	7.98 ± 1.02	>300
5	R = R′ = carbonyl	>80.0	n.d.
6	R = NH_2, R′ = H, racemic	15.7 ± 1.4	n.d.
7	R = $NHCH_2CH_2NH_2$, R′ = H, racemic	7.38 ± 0.22	>300
8	R = piperazine, R′ = H, racemic	>50.0	n.d.

Table 4
IC_{50} values of bis-carbazole or bis-napthyl analogues with various linkers

Compound	Structure	BACE-1 IC_{50} (μM)	Cathepsin D IC_{50} (μM)
12		0.71 ± 0.10	>300
13		>30	n.d.
14		>100	n.d.
15		8.82 ± 0.50	>300

Also it can be inferred that the linker chain length of four methylene units is slightly better than three methylene units.

Finally, a set of compounds was prepared, in which the 2-propanol linker was substituted in 1- and 3-positions with two carbazole or two 1-napthylamine moieties. Surprisingly, the bis-carbazole **12** was very potent with an IC_{50} of 710 nM, while poor inhibition was determined for bis-napthyl **13** (Table 4). Consequently, analogues of **12** with 4 carbon linker, **14** and **15**, were prepared to probe the optimal linker length and the position of the alcohol group, but they did not improve on the inhibition of BACE-1 over **12** with 3 carbon linker.

Besides potency against BACE-1 and selectivity over off-target proteases such as cathepsin D, useful inhibitors must also have acceptable physicochemical properties to penetrate the blood–brain barrier. A selection of BACE-1 inhibitors has been evaluated for blood–brain barrier permeability according to the rules proposed by Clark and Lobell (Table 5).[30,31] These rules predict that a compound will possess acceptable blood–brain barrier permeability if the compound's molecular weight is less than 450 g/mol, the polar surface area (PSA) <60–70 $Å^2$, the number of nitrogens and oxygens (N + O) <6, and the parameter *c*log*P*-(N + O) >0. Alternatively, using QikProp,[32] the partition coefficient log BB, which is defined as the ratio of the steady-state concentration of the compound in the brain over the one in the blood ($\log BB = \log([\text{compound}]_{brain}/[\text{compound}]_{blood})$), has been computationally calculated for the most promising compounds. Excellent blood–brain permeabilities are predicted for compounds with a log BB >0.3, while compounds with a log BB <−1.0 are classified to possess very poor blood–brain barrier permeability.[32,33] Analysis of the most potent compounds against BACE-1 revealed that all analogues are in compliance with the rules dictated by Clark and Lobell. Furthermore, log BB predictions clearly propose that the blood–brain barrier permeability is in the acceptable range for the most potent compounds such as **E7T18**, **9**, and **12**. Compound **E8T18**, a close analogue of **E7T18** in which the carbazole ring has been replaced by the 2-phenyl-indole moiety, has excellent calculated blood–brain barrier permeability. Overall, these predictions are encouraging and underline the potential of the herein described compound series.

Table 5
Physicochemical parameters to predict the compound's potential to penetrate the blood–brain barrier

Compound	MW	N + O	PSA	*c*log*P*–(N + O)	log BB
E7T18	383.5	2	20.4	4.53	−0.013
E8T18	409.5	2	21.0	5.25	0.374
E7T3	351.4	2	20.4	3.55	0.091
2	367.5	1	2.81	6.85	0.287
9	397.5	2	20.9	4.84	−0.080
10	397.5	2	20.5	4.96	0.005
11	413.5	3	38.6	3.44	−0.285
12	390.5	3	23.2	3.85	0.009
15	420.5	4	46.3	2.84	0.091

All parameters have been calculated using the software QikProp (Schrödinger, LLC, New York).[32]

In summary, we have conducted a study on a compound series of 1,3-disubstituted 2-propanol for the inhibition of BACE-1. A selected set of 121 compounds has been combinatorially prepared and tested for inhibition of BACE-1 activity and hit compounds such as **E7T18, E7T3** and **E8T18** have been identified to have IC_{50}s of 4.93, 5.36 and 6.66 μM, respectively. For the first time, the selectivity of 1,3-disubstituted 2-propanols to inhibit BACE-1 over other essential aspartyl protease was tested. Promising hit compounds have demonstrated to selectively inhibit BACE-1 over cathepsin D with a selectivity factor of 50 or more. Subsequently, structure–activity relationship studies focusing primarily on analogues of hit compound **E7T18** have been undertaken to further refine the initial hit compounds. The screening results including the detailed structure–activity relationship studies suggest that best BACE-1 inhibition is observed if (a) a naphthyl or carbazole ring in combination with an extended aromatic ring system such as a carbazole or a 2-phenyl-indole is present to occupy the S1, S2, S1′, and S2′ subpockets, (b) the linker has four methylene units, which is marginally better than the linker with three units, (c) the linker is sterically compact and it can accommodate only one hydroxyl group. Further optimization efforts ultimately lead to the most potent and selective compound, bis-carbazole **12**, with an IC_{50} of 710 nM against BACE-1 and a selectivity of >422 times over cathepsin D.

The structure–activity relationship data with respect to the hydroxyl-containing linker supports the docking model proposed by Macchia for HEA compound series.[19] Additional studies are required, however, to fully understand the details of how compounds such as hit **E7T18** or bis-cabazole **12** interact with BACE-1 on an atomic-level resolution. Nonetheless, the good potency in conjunction with high selectivity over cathepsin D and good blood–brain barrier permeability predictions make the herein presented

compound series an excellent platform for future development of potent and selective BACE-1 inhibitors.

Experimental section

Compounds were prepared using synthetic procedures as reported in the Supplementary data. The purity of all the compounds tested for BACE-1was found to be $\geqslant$95% via HPLC analysis. Assay for BACE-1 has been done using previously reported methodology.[24–26] Cathepsin D activity has been done using FRET-based assay kits from Anaspec (catalog no. 72170), which was validated by testing with known inhibitor of Cathepsin D. For the testing of compounds against BACE-1 and cathepsin D, measurements were made in triplicates and inhibitory concentrations IC_{50}s are reported with the standard deviation.

Funding

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Supplementary data

Supplementary data (experimental details of the synthesis for all compounds including ^{1}H NMR, ^{13}C NMR, and HR-MS for all tested compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.072.

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