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Structure-based design, synthesis, and biological evaluation of dihydroquinazoline-derived potent β-secretase inhibitors

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ABSTRACT

Structure-based design, synthesis, and biological evaluation of a series of dihydroquinazoline-derived β-secretase inhibitors incorporating thiazole and pyrazole-derived P2-ligands are described. We have identified inhibitor **4f** which has shown potent enzyme inhibitory (K_i = 13 nM) and cellular (IC_{50} = 21 nM in neuroblastoma cells) assays. A model of **4f** was created based upon the X-ray structure of **3a**-bound β-secretase. The model suggested possible interactions in the active site.

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β-Secretase (BACE1, memapsin 2) is an important molecular target for the treatment of Alzheimer's disease (AD).[1] This membrane-bound aspartic protease catalyzes the cleavage of β-amyloid precursor protein (APP) leading to the formation of amyloid-β-peptide (Aβ) in the brain. The neurotoxicity of Aβ leads to brain inflammation, neuronal death, dementia, and AD.[2,3] Early on, we designed a potent substrate-based transition-state inhibitor **1**.[4] An X-ray structure of **1**-bound β-secretase led to the structure-based design of a series of BACE1 inhibitors.[5] Over the years, we and others have designed a variety of BACE1 inhibitors incorporating novel peptidomimetic and nonpeptide scaffolds.[6–8] A number of BACE1 inhibitors have been shown to reduce Aβ-levels in transgenic AD mice. Furthermore, we recently showed that administration of β-secretase inhibitor GRL-8234 (**2**) rescued the decline of cognitive function in transgenic AD mice.[9] While the development of a clinically effective inhibitor has not yet emerged, important progress has been made with small-molecule inhibitors belonging to different structural classes.[1]

In 2007, Baxter and co-workers reported an interesting class of 2-amino-dihydroquinazoline-derived BACE1 inhibitors.[10] A representative example is inhibitor **3a** (Fig. 1) with a reported K_i of 11 nM. An X-ray structure of **3a**-bound β-secretase provided the important molecular interactions responsible for its high affinity.[10] Based upon this reported X-ray structure, we have designed a

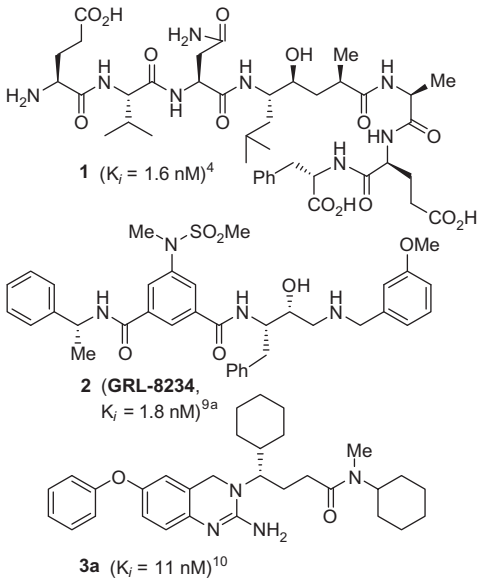

Figure 1. Structures of inhibitors **1**–**3**.

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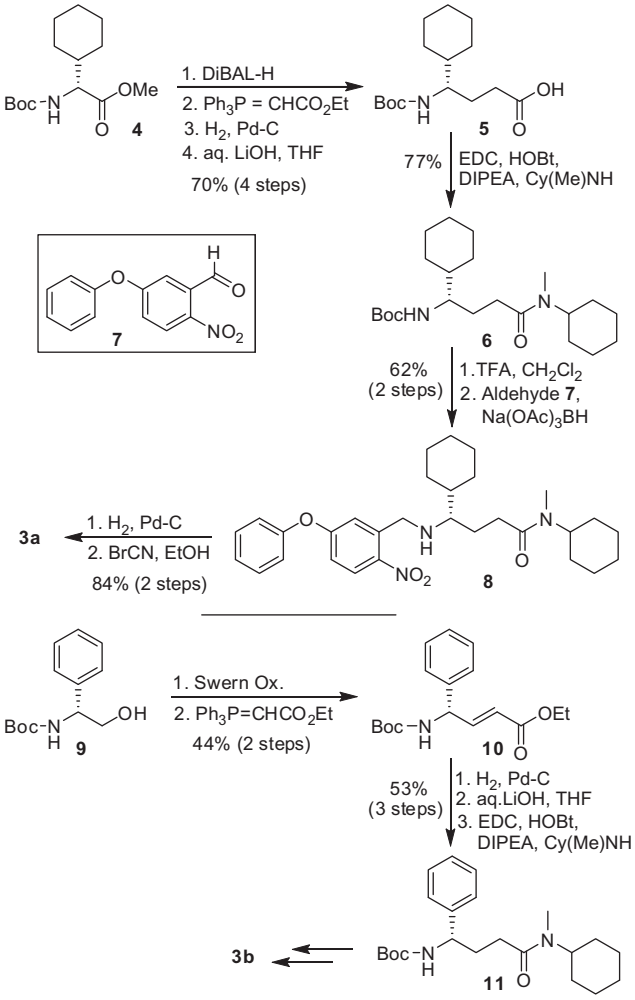
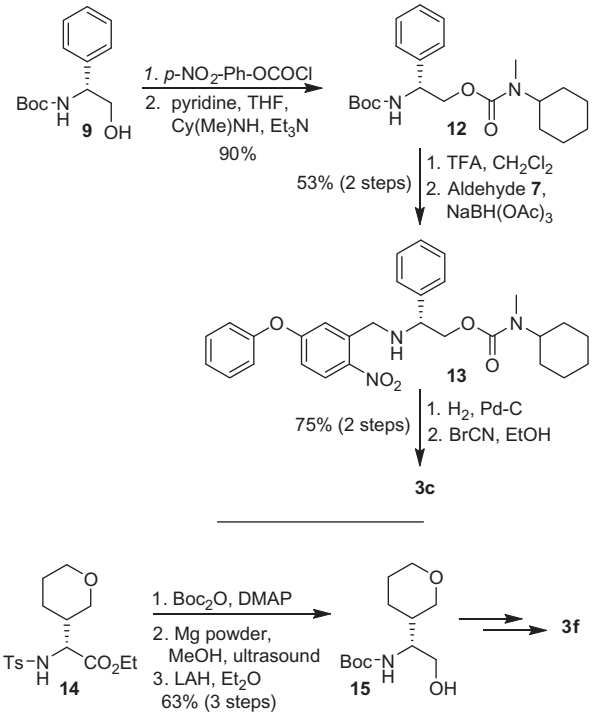


Scheme 1. Synthesis of dihydroquinazoline-derived BACE1 inhibitors.



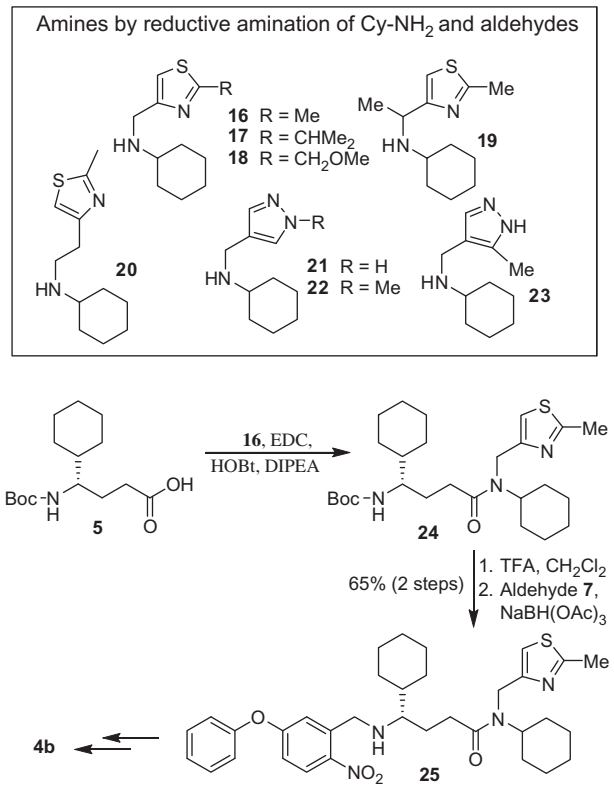
Scheme 2. Synthesis of urethane-derived inhibitors.

number of functionalities and heterocyclic scaffolds, to make specific interactions in the β-secretase active site to improve enzyme affinity and potency. Herein, we report the design, synthesis, and evaluation of a series of potent 2-amino-dihydroquinazoline-derived inhibitors incorporating urethanes and heterocycles such as pyrazoles and thiazoles. A number of compounds exhibited potent β-secretase inhibitory activity and displayed good potency in cellular assays. To obtain molecular insight into the possible ligand-binding site of β-secretase, we have created an energy-minimized model of **4f** based upon the **3a**-bound β-secretase X-ray structure.

The synthesis of various dihydroquinazoline derivatives is shown in Scheme 1. The Boc-protected (*R*)-cyclohexylglycine methyl ester **4** was reduced by DIBAL-H at −78 °C and the resulting aldehyde was reacted with (ethoxycarbonylmethylene)triphenylphosphorane in a mixture of CH_2Cl_2 and methanol to provide the corresponding α,β-unsaturated ester. The ester was subjected to hydrogenation over Pd–C for 12 h to provide the corresponding saturated derivative. Saponification of the ester with aqueous LiOH afforded the acid **5** in 70% overall yield. For the synthesis of quinazoline derivative **3a**, acid **5** was coupled with *N*-cyclohexylmethyl amine to provide amide **6** in 77% yield. Removal of the Boc-group was carried out by treatment with trifluoroacetic acid and the resulting amine was subjected to reductive amination with aldehyde **7**[10,11] to afford nitro compound **8** in 62% yield. Hydrogenation of **8** over 10% Pd–C in ethyl acetate at 23 °C provided the corresponding amine. Reaction of this resulting amine with BrCN in ethanol at reflux furnished dihydroquinazoline derivative **3a** in 84% yield. For the synthesis of inhibitor **3b**, (*R*)-phenylglycinol derivative **9** was oxidized under Swern conditions and the resulting aldehyde was reacted with (ethoxycarbonylmethylene)triphenylphosphorane in THF to provide α,β-unsaturated ester **10**. Hydrogenation of the ester over 10% Pd–C in ethyl acetate and saponification of the ester provided the corresponding acid which was coupled with *N*-cyclohexylmethyl amine to provide amide **11** in 53% overall yield. Amide **11** was converted to dihydroquinazoline derivative **3b** by following the same sequence of reactions as **3a**.

We have investigated the potential of various urethane derivatives as BACE1 inhibitors. A representative synthesis of urethane derivative **3c** is shown in Scheme 2. Phenylglycinol derivative **9** was reacted with 4-nitrophenylchloroformate to provide the corresponding mixed carbonate.[12] Reaction of *N*-cyclohexylmethyl amine with this mixed carbonate afforded urethane derivative **12** in 90% yield in two steps. Removal of the Boc-group with trifluoroacetic acid and reductive amination of the resulting amine with aldehyde **7** furnished nitro derivative **13** in 53% yield, in two steps. Hydrogenation of nitro compound **13** over 10% Pd–C in ethyl acetate followed by exposure of the resulting amine to BrCN afforded inhibitor **3c**. Urethane derivatives **3d** and **3e** were prepared by an analogous procedure using Boc-protected (*R*)-cyclohexyl glycinol as the starting material. For synthesis of urethane **3f**, racemic amino ester **14** was prepared using a multicomponent reaction developed in our laboratory.[13] Reaction of **14** with Boc_2O in the presence of DMAP in CH_3CN at 23 °C afforded the corresponding Boc-derivative. Treatment of the resulting ester with magnesium powder in methanol under sonication afforded the corresponding Boc-amino ester.[14] Reduction of the resulting ester with LAH afforded the racemic alcohol **15** in 63% overall yield. This alcohol was converted to urethane derivative **3f** and amide derivative **3g** as described above.

Based upon the X-ray structure of **3a**-bound β-secretase, we planned to incorporate various heterocyclic derivatives in place of the methyl group in **3a** to interact with the β-secretase active site. We were particularly interested in designing inhibitors with thiazole and pyrazole derivatives since these heterocycles contain

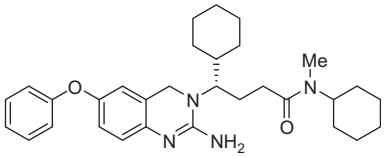
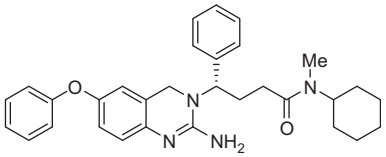
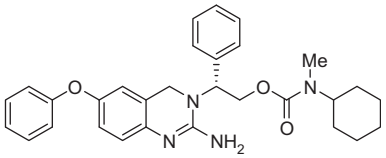
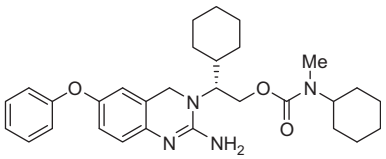
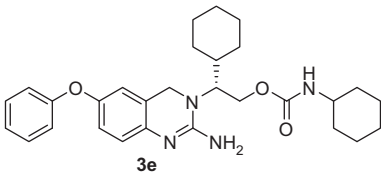
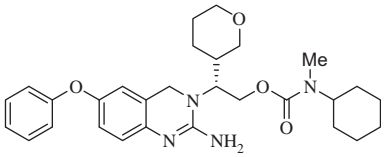
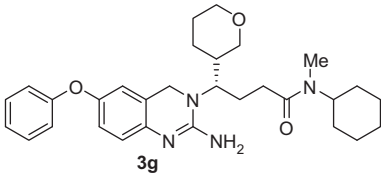


Scheme 3. Synthesis of memapsin 2 inhibitors.

hydrogen bond donor and acceptor groups for interactions in the enzyme active site. Also, these heterocycles are inherent to numerous bioactive natural products and FDA approved therapeutic agents.[15,16] Accordingly we designed a number of inhibitors containing thiazole and pyrazole derivatives. The synthesis of various dihydroquinazoline derivatives are shown in Scheme 3. *N*-cyclohexyl derivative **16** was prepared by reductive amination of the corresponding 4-thiazolecarboxaldehyde[17] and cyclohexyl amine. Similarly, amines **17**–**20** were prepared from the corresponding aldehydes.[18–21] Various known pyrazole aldehydes[22] were converted to amines **21**–**23** by reductive amination with cyclohexylamine. For synthesis of dihydroquinazoline **4b**, acid **5** was coupled with amine **16** in the presence of EDC, and HOBt to provide amide **24**. Removal of the Boc-group and subsequent reductive amination with aldehyde **7** furnished nitro derivative **25** in 65% overall yield. This was converted to quinazoline **4b** as described above. Other inhibitors **4c**–**4i** were prepared following a similar protocol as **4b**.

The β-secretase inhibitory activity of various inhibitors was determined against recombinant β-secretase using our previously reported assay protocols.[23] The results are shown in Table 1. As can be seen, our synthetic dihydroquinazoline derivative **3a** has shown K_i value of 25 nM. The corresponding phenyl derivative **3b** exhibited nearly a sixfold lower enzyme inhibitory potency. We have then investigated the corresponding urethane derivative **3c**. However, this inhibitor displayed nearly a fivefold loss of potency compared to **3b**. However, the corresponding cyclohexyl urethane derivative **3d**, improved potency by 20-fold over **3c** (entry 4). The presence of a methyl group is important as the cyclohexyl urethane derivative **3e** is less potent. We have also incorporated a tetrahydropyran ring in place of the cyclohexyl group in **3d**. As shown, racemic mixture (1:1) **3f** has shown reduced potency over cyclohexyl derivative **3d**. We have also examined the effect of a ring oxygen in carboxamide derivative **3g**. This derivative too lost

Table 1
Enzyme inhibitory and cellular activity of inhibitors

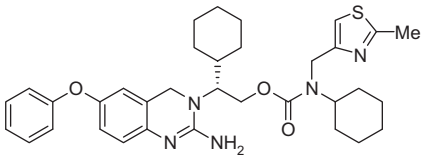
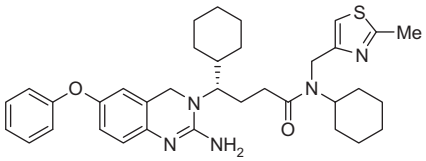
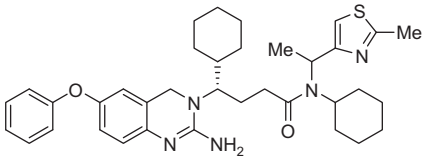
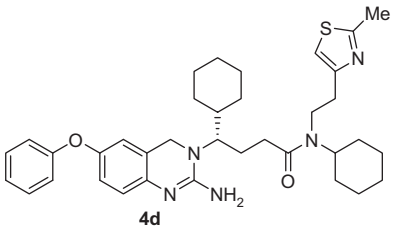
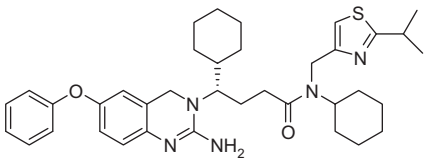
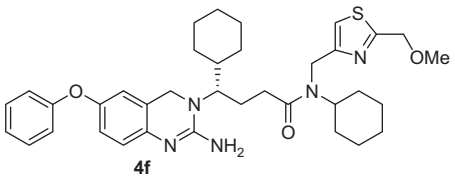
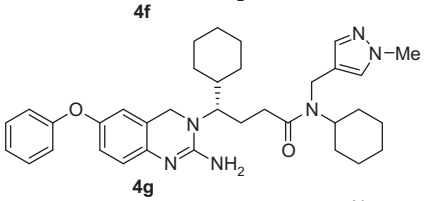
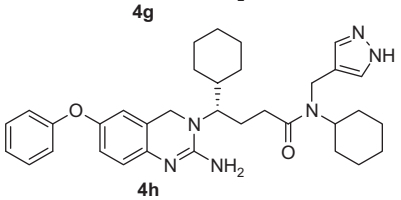
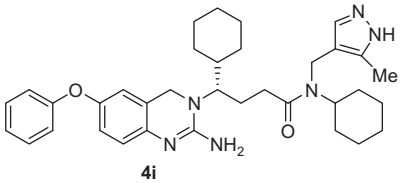
Entry	Inhibitor	K_i (nM)	IC_{50}[a,b] (nm)
1.	 **3a**	25	71
2.	 **3b**	163.8	482
3.	 **3c**	783	3400
4.	 **3d**	37.5	nt[c]
5.	 **3e**	60	nt
6.	 **3f**	104	1100
7.	 **3g**	122	nt

[a] IC_{50} was determined in neuroblastoma cells.
[b] GRL-8234 exhibited K_i = 1.8 nM, IC_{50} = 2.5 nM in this assay.[9a]
[c] Not tested.

nearly fivefold potency compared to cyclohexyl derivative **3a**. We have also evaluated the cellular inhibition of β-secretase in neuroblastoma cells.[24] Inhibitor **3a** has shown an average cellular IC_{50} value of 71 nM. The corresponding phenyl derivative displayed an IC_{50} of 482 nM. The urethane derivative **3c** was significantly less potent compared to inhibitor **3b**. Similarly, urethane derivative **3f** showed an IC_{50} value of 1.1 μM (entry 6).

Based upon the reported X-ray structure of **3a**-bound β-secretase, we have incorporated various thiazole and pyrazole-derived ligands in an effort to interact with residues in the β-secretase active site. As can be seen in Table 2, incorporation of a (2-methylthiazol-4-yl)methyl substituent in place of the methyl group in **3d**, resulted in nearly a 40-fold loss of enzyme inhibitory activity (entry 1). The corresponding amide derivative **4b** also exhibited a loss of

Table 2
Enzyme inhibitory and cellular activity of inhibitors

Entry	Inhibitor	K_i (nM)	IC_{50}[a, b] (nm)
1.	 **4a**	1463	nt[c]
2.	 **4b**	79	390
3.	 **4c**	104	nt
4.	 **4d**	106	nt
5.	 **4e**	144	nt
6.	 **4f**	13	21
7.	 **4g**	11	23
8.	 **4h**	23	48
9.	 **4i**	39	51

[a] IC_{50} was determined in neuroblastoma cells.
[b] GRL-8234 exhibited K_i = 1.8 nM, IC_{50} = 2.5 nM in this asay.[9a]
[c] Not tested.

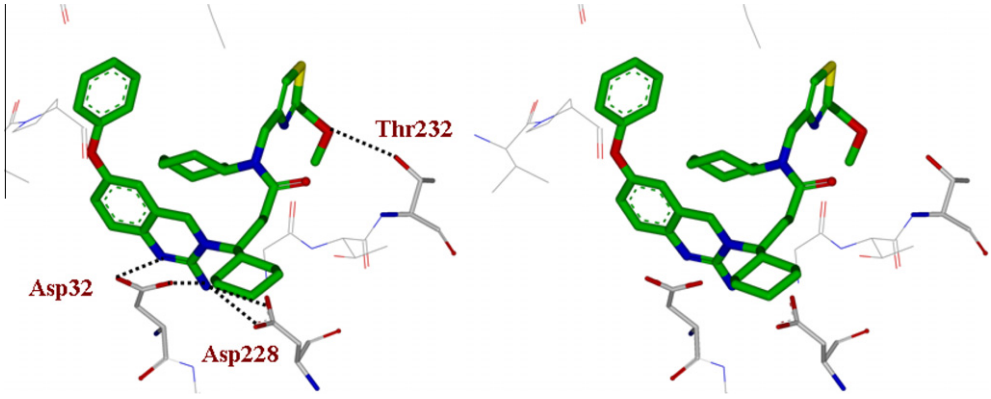


Figure 2. Stereoview of the model of inhibitor **4f** (green carbon) with β-secretase. Possible hydrogen bonds between the inhibitor and β-secretase are shown in black dotted lines.

potency over **3a** (entry 2). Interestingly, this compound displayed poor cellular β-secretase activity in neuroblastoma cells over **3a**. Introduction of a methyl group on the methylene side chain in **4b** resulted in **4c** which showed a loss of potency (entry 3). Furthermore, chain elongation in **4d** and incorporation of (2-isopropylthiazol-4-yl)methyl substituent in **4e** did not improve potency (entries 4 and 5). Interestingly, incorporation of a methoxymethyl substituent on the thiazole side chain in **4f** resulted in nearly a sixfold improvement of enzyme activity over **4b**. Furthermore, inhibitor **4f** has shown very potent cellular inhibitory properties in neuroblastoma cells (entry 6). We have also investigated various substituted pyrazolylmethyl groups (entries 7–9). Methyl substituted pyrazole derivative **4g** is more potent than the unsubstituted derivative **4h** in both enzyme inhibitory and cellular assays. Incorporation of methyl group on the pyrazole ring in **4i** did not improve potency over the N-alkylated or unalkylated derivatives.

To gain insight into specific ligand-binding site interactions, an energy minimized model structure of **4f** was created in the active site of BACE1, based upon the crystal structure of **3a**-bound β-secretase.[10] The conformation of **4f** was optimized using the CHARMM force field.[25] As shown in Figure 2, 2-amino dihydroquinazoline functionality forms a unique hydrogen bonding network with the catalytic aspartic acids Asp32 and Asp228 and the (*S*)-cyclohexyl group nicely filled in the S1′-subsite as reported in the X-ray structure.[10] The 2-methoxymethylthiazole moiety appears to fill in the hydrophobic pocket in the S2-subsite. Furthermore, the methoxy oxygen is within proximity to form a hydrogen bond with Thr232. This may explain the sixfold enhancement of enzyme inhibitory potency over the 2-methylthiazole derivative **4b**. The P1-cyclohexamide fits well into the S1-site of β-secretase.

In summary, we have carried out structure-based modifications of 2-amino-3,4-dihydroquinazoline-derived β-secretase inhibitors. In particular, we have incorporated thiazole and pyrazole-based P2-ligands to make specific interactions in the S2-subsite. These efforts resulted in inhibitors with improved potency and cellular inhibitory properties compared to methyl-substituted inhibitor **3a**. Inhibitor **4f** has shown enhanced enzyme inhibitory activity as well as very good cellular inhibitory potency in neuroblastoma cells. A protein-ligand X-ray structure-based model of **4f**-bound β-secretase has provided important molecular insight into the ligand-binding site interactions. Further design and improvement of inhibitor properties are currently in progress.

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