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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 revealed critical interactions in the active site.

Keywords

β-Secretase; Alzheimer's Diseas; Memapsin 2; Inhibitor; Design and Synthesis; Dihydroquinazoline

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 GRL8234 (**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**

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(Figure 1) with reported a 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 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 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 Boc 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 dihydroquinzoline derivative **3a** has shown K_i value of 25 nM. The corresponding phenyl derivative **3b** exhibited nearly a 6-fold lower enzyme inhibitory potency. We have then investigated the corresponding urethane derivative **3c**. However, this inhibitor displayed nearly a 5-fold 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 methyl group is important as the cyclohexyl urethane derivative **3e** is less potent. We have also incorporated 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 nearly 5-fold 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 (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 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 6-fold 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 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 a 6-fold 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.

Acknowledgments

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References and Notes

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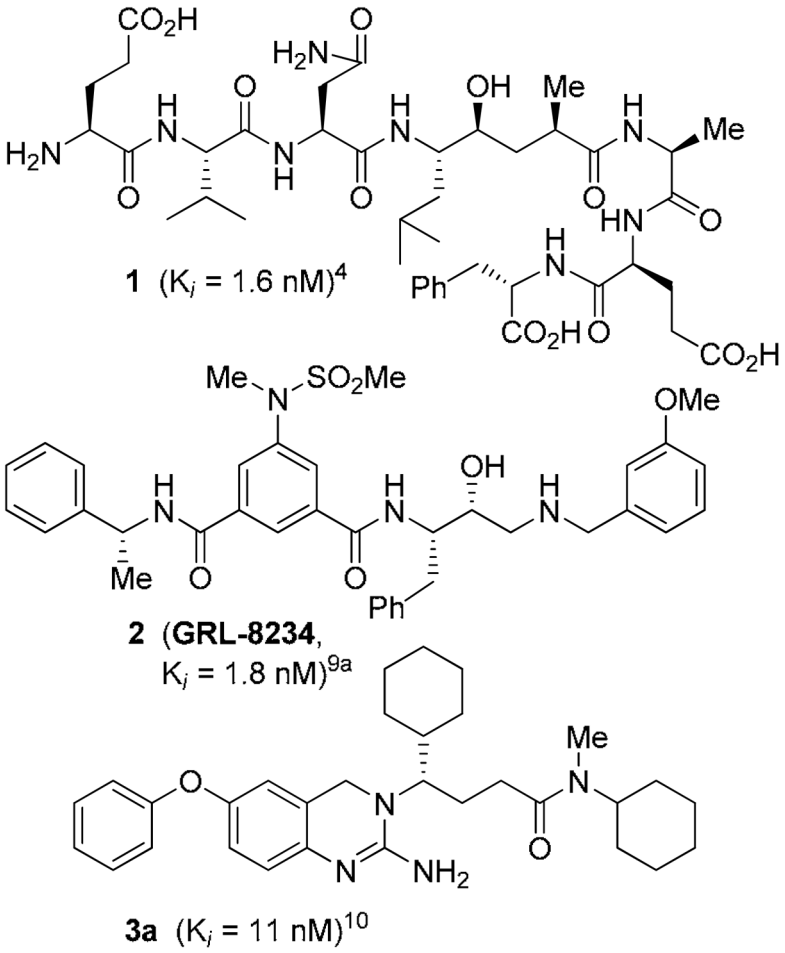


Figure 1.
Structures of inhibitors **1**–**3**

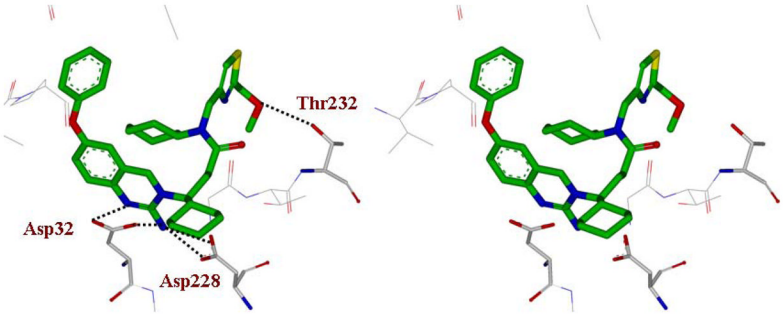
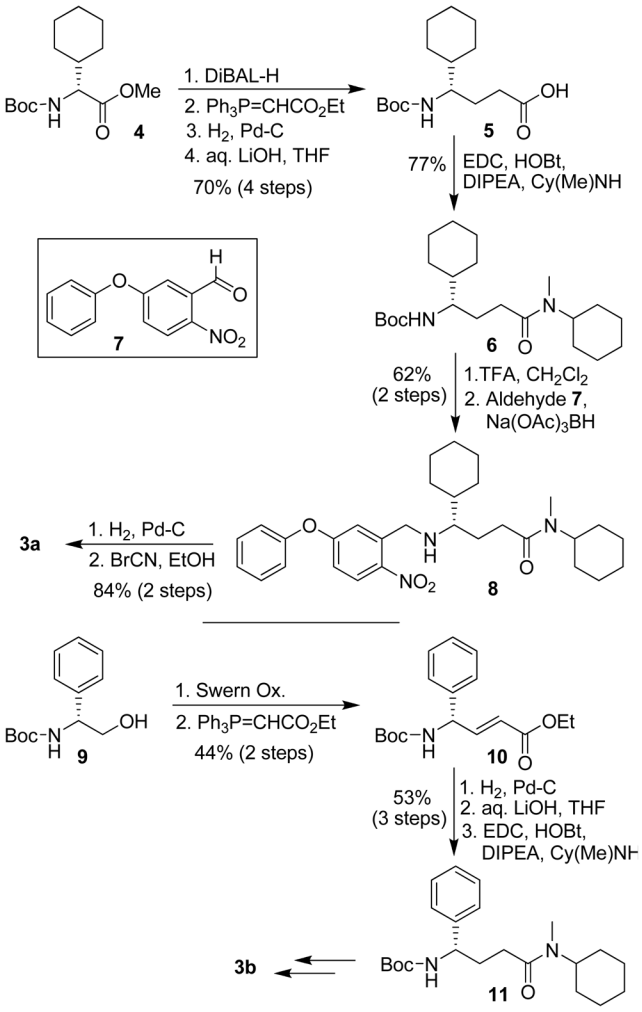
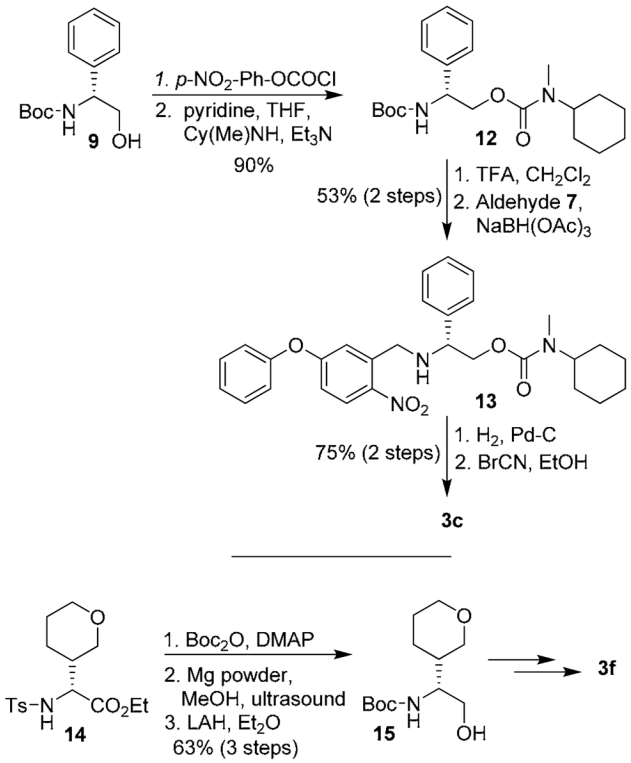


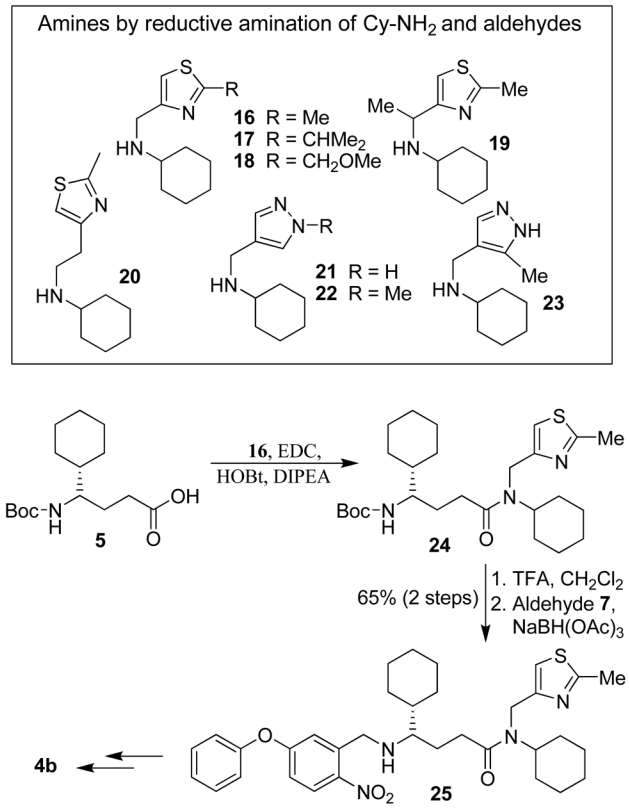
Figure 2.
Stereoview of the model of inhibitor **4f** (green carbon) with β-secretase. Possible hydrogen bonds between the inhibitor and β-secretase are shown in black dotted lines.



Scheme 1.
Synthesis of dihydroquinazoline-derived BACE1 inhibitors



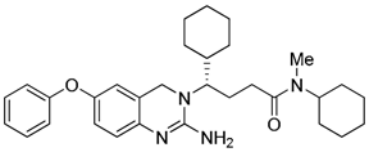
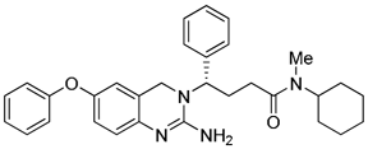
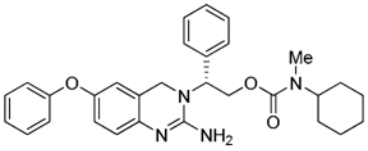
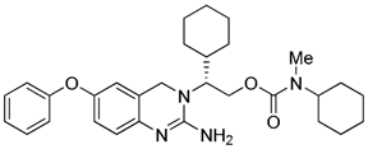
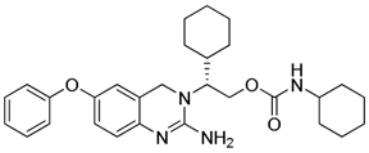
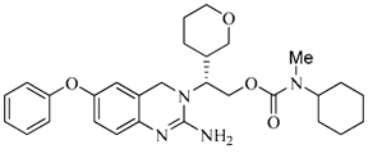
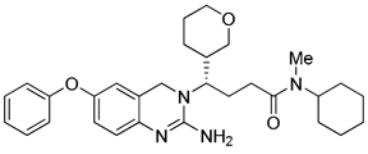
Scheme 2.
Synthesis of urethane-derived inhibitors



Scheme 3.
Synthesis of memapsin 2 inhibitors

Table 1

Enzyme inhibitory and cellular activity of inhibitors

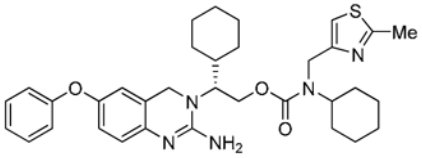
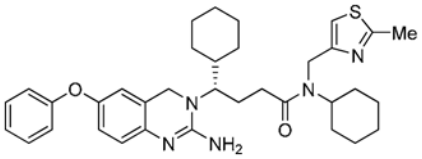
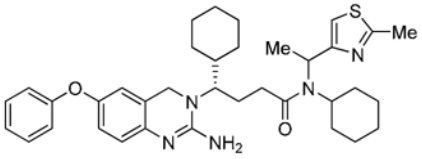
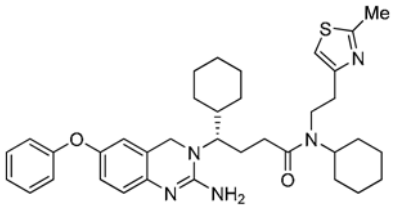
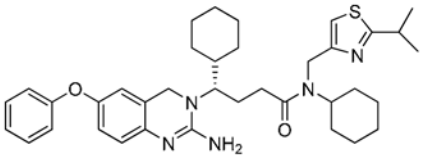
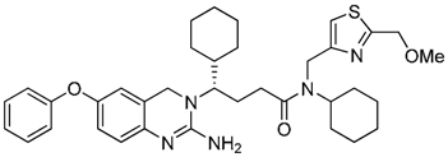
Entry	Inhibitor	k_i (nM)	IC_{50}(nM)[a,b]
1.	 **3a**	25	71
2.	 **3b**	163.8	482
3.	 **3c**	783	3400
4.	 **3d**	37.5	nt
5.	 **3e**	60	nt
6.	 **3f**	104	1100
7.	 **3g**	122	nt

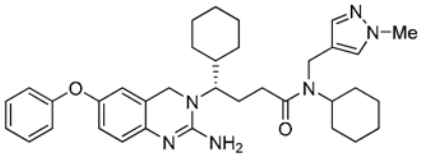
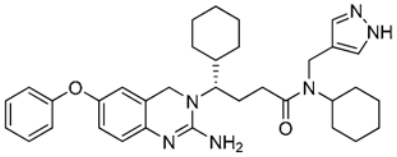
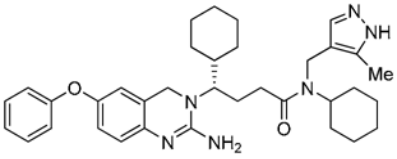
[a] IC_{50} was determined in neuroblastoma cells.

[b] GRL-8234 exhibited K_i = 1.8 nM, IC_{50} = 2.5 nM in this asay.[9a]

Table 2

Enzyme inhibitory and cellular activity of inhibitors

Entry	Inhibitor	k_i (nM)	IC_{50}(nM)[a,b]
1.	 **4a**	1463	nt
2.	 **4b**	79	390
3.	 **4c**	104	nt
4.	 **4d**	106	nt
5.	 **4e**	144	nt
6.	 **4f**	13	21

Entry	Inhibitor	k_i (nM)	IC_{50}(nM)[a,b]
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 assay.[9a]