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Triazole-linked reduced amide isosteres: An approach for the fragment-based drug discovery of anti-Alzheimer's BACE1 inhibitors

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ABSTRACT

In the course of a β-site APP-cleaving enzyme 1 (BACE1) inhibitor discovery project an in situ synthesis/screening protocol was employed to prepare 120 triazole-linked reduced amide isostere inhibitors. Among these compounds, four showed modest (single digit micromolar) BACE1 inhibition. Our ligand design was based on a potent reduced amide isostere **1**, wherein the P_2 amide moiety was replaced with an anti-1,2,3-triazole unit. Unfortunately, this replacement resulted in a 1000-fold decrease in potency. Docking studies of triazole-linked reduced amide isostere **A3Z10** and potent oxadiazole-linked tertiary carbinamine **2a** with BACE1 suggests that the docking poses of **A3Z10** and **2a** in the active sites are quite similar, with one exception. In the docked structures the placement of the protonated amine that engages D228 differs considerably between **2a** and **A3Z10**. This difference could account for the lower BACE1 inhibition potency of **A3Z10** and related compounds relative to **2a**.

Alzheimer's disease (AD) is a progressive neurodegenerative disease whose first clinical manifestation is cognitive impairment, followed by dementia, and eventually death. Data from the 2000 US census indicates that AD currently affects more than 4.5 million Americans.[1] According to the Alzheimer's Association, this affliction is the 6th leading cause of death in the United States.[2] The amyloid cascade hypothesis postulates that AD results from an accumulation of the 40 and 42 amino acid residue peptides, Aβ or Aβ40/42, which form insoluble aggregates. These aggregates or plaques are implicated in subsequent neurodegenerative processes that lead to cognitive impairment.[3] Since formation of Aβ requires proteolytic cleavage of the amyloid precursor protein (APP) by the β-site APP cleaving enzyme 1 (BACE1), BACE1 appears to be a logical target to slow or halt the progression of AD.[4]

Coburn et al. at Merck reported that reduced amide **1** exhibited 8 nM potency to inhibit BACE1 (Fig. 1).[5] We sought to further reduce the peptidic nature of **1** by replacing the P_2 amide (boxed) with a 1,2,3-triazole heterocycle. 1,2,3-Triazoles have been shown to be effective amide surrogates in applications such as acetylcholinesterase inhibitors[6] and HIV-1 protease inhibitors.[7]

The large dipole moment, combined with the ability of the sp^2 nitrogen atoms to serve as hydrogen bond acceptors, make the 1,2,3-triazole an appealing amide mimic.[7] The potent Merck inhibitor **2a**[8] replaced the P_2 amide of **1** with an oxadiazole, simultaneously exchanging the reduced amide isostere with a tertiary carbinamine. It thus seemed possible that simple anti-triazole replacement of the P_2 amide in **1** might also be tolerated.

An X-ray crystal structure of **2a** bound to BACE1 (PDB ID: 2IRZ) demonstrated that the oxadiazole nitrogens mimic the P_2 amide carbonyl in receiving an H-bond from an important residue (Q73) within the active site of the BACE1 enzyme (PDB ID 2IRZ),[8] and it seemed likely that the nitrogen atoms of a 1,2,3-triazole could play the same role. Additionally, the incorporation of a 1,2,3-triazole unit would allow facile inhibitor assembly via the copper-catalyzed acetylene azide cycloaddition reaction (CuAAC); in this regard we wanted to apply the high throughput screening (copper-catalyzed microtiter plate screening) technique of Wong, Sharpless, and Fokin.[9,10] In this way we hoped to rapidly interrogate both subtle and major P- and P′-side changes in the structure of triazole-linked analogs of **1**. Note that after we had concluded our work, a Merck team in 2010 reported a 1,2,3-triazole-linked tertiary carbinamine inhibitor **2b**, which surprisingly offered dramatically reduced inhibition potency relative to **2a** (16.3 vs 0.012 μM, respectively).[11] We offer further commentary on the relative potencies of **2a** and **2b** in the modeling section below.

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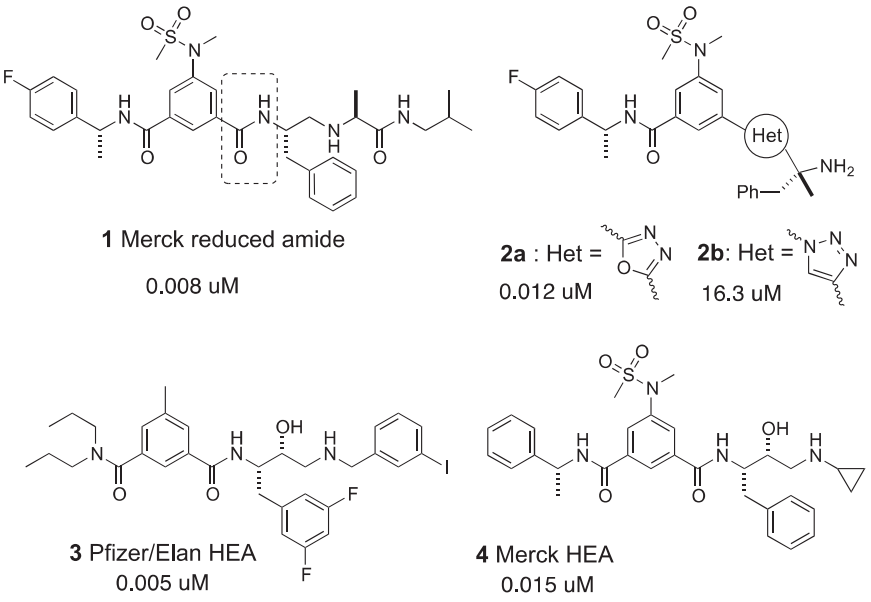


Figure 1. Reduced amide 1, heterocycle-linked tertiary carbinamines **2a** and **2b**, and HEA 3–4 inhibitors of BACE1.

Our approach differed from that of Merck's triazole **2b** in two ways (Fig. 2). Firstly, the warhead of the inhibitor would incorporate a reduced amide isostere rather than a tertiary carbinamine. Secondly, we decided to synthesize acetylene fragments that comprise the P_3–P_2 (i.e., left) portion of **1**, and use the azide fragments to incorporate the reduced amide P_1–$P_{2'}$ fragment (Figure 2). Note that triazole **2b** is formally derived from a P_3–P_2 azide and a P_1–$P_{1'}$ acetylene.

We were cognizant that our synthetic strategy would increase the distance between the isophthalimide and the basic amine by one bond relative to **1** and **2a**, but believed this change would not necessarily be deleterious, given the planned variation at P_3, P_1, $P_{1'}$ and $P_{2'}$. In retrospect we can note that triazole-linked tertiary carbinamine **2b**, which maintains the spacing of oxadiazole **2a**, nevertheless lost significant inhibition potency relative to **2a**.

The acetylenes employed are depicted in Figure 3. Acetylenes **A1**–**A4** were designed to mimic the isophthalimide P_3–P_2 portion of **1**, **2a**, **3**, and **4**; their syntheses are described in Supplementary data. Acetylenes **A5**–**A10** were gifts and were incorporated in the screen to search for new ligands for the P_3–P_2 region. We have also recently reported regioselective syntheses of **A10a** (C_i-symmetric) and (±)-**A10b**.[12] Since 1,4-diethynyl benzene is bifunctional and could make triazole dimers, for our screening experiments we gave it two different designations based on the equivalents used for the CuAAC reaction (**A8** 1 equiv; **A9** 3.6 equiv).

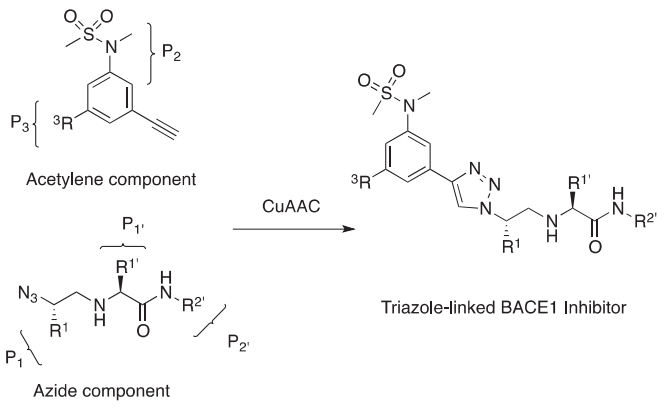


Figure 2. Synthetic strategy employed for 1,2,3-triazole-linked reduced amide inhibitors.

The selected azide components **Z1**–**Z12** are shown in Scheme 1. Azides **Z1**–**Z8** incorporate the $P_{1'}$ amide functionality of Merck reduced amide **1**, and feature the favored benzyl (cf. **1**–**4**) and isobutyl[13,14] groups as P_1 substituents. Azides **Z9**–**Z12** replace the $P_{1'}$ amide with a benzylamine group, a strategy that was successful for inhibitor **3**[15] and other similar HEA isosteres.[14] Two key transformations were used in the syntheses of azides **Z1**–**Z12**. The first was an oxidation/reductive amination sequence of **5** and **6** to give the secondary amines **7a**–**d** and **8a**–**d**, and **9** to give secondary amines **10a**–**d** (Scheme 1). Following deprotection of the Cbz or Boc groups of **7**–**9**, respectively, the substrate contained two amine groups. As hoped, we found that only the primary amines reacted in the second key transformation (diazotransfer), affording azides **Z1**–**Z12** in moderate to good yields. This finding is consistent with the mechanism proposed by Nyffeler et al.[16] but to our knowledge this is the first example of the diazotransfer reaction performed in the presence of a secondary amine.

With the acetylene and azide building blocks in hand we began screening an array of triazole-linked reduced amide isosteres using an in situ synthesis/assay protocol. We based our protocol on the Cu-catalyed microtiter plate-based synthesis/screening procedure of Brik et al. making minor modifications.[9,17] All 120 possible combinations of **A1**–**A10** with **Z1**–**Z12** were prepared and assayed in this way, using the commercially available HEA isostere inhibitor **4**[18] (Inhibitor IV, Calbiochem product catalog no. 565788) as a positive control. In addition to **4**, three of the 120 binary combinations showed significant inhibition at 10 μM: **A3Z9**, **A3Z10**, and **A10Z10**. These triazoles were then synthesized by traditional organic techniques, purified, and assayed. Since **A10** was a mixture of regioisomers, these were separated (the 1,4-diol was designated **A10a** and the 1,3-diol **A10b**) and were used in the CuAAC reaction with **Z10** to afford **A10aZ10** and **A10bZ10**, both as mixtures of diastereomers. Unfortunately, these compounds only showed modest, single digit micromolar inhibition of BACE1 (Table 1), similar to that of Merck triazole-linked tertiary carbinamine **2b**. In contrast, HEA inhibitor **4** exhibited an IC_{50} of 0.020 μM, similar to the literature report.[18]

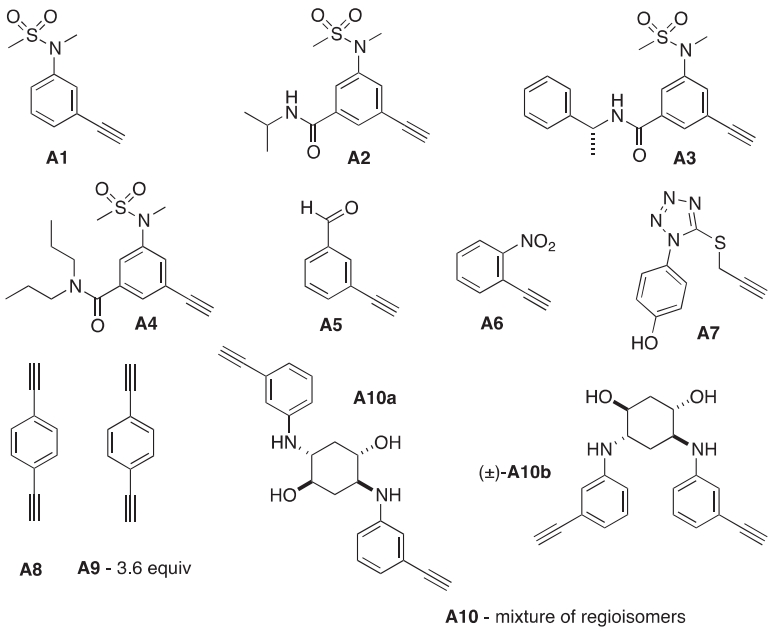
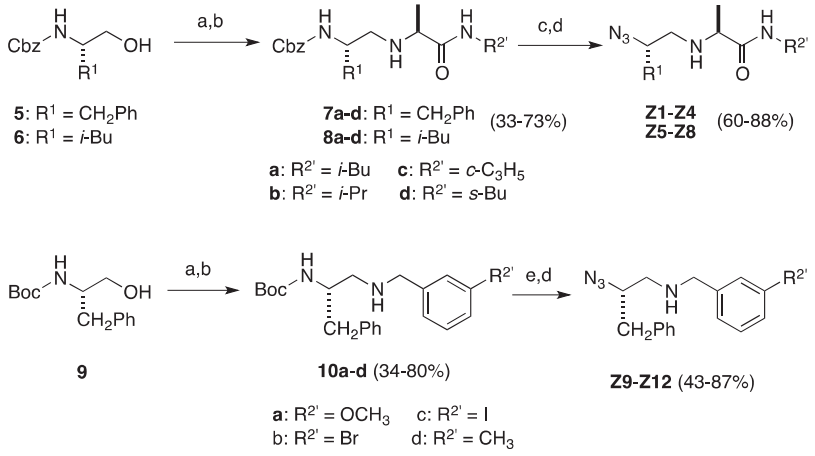


Figure 3. Acetylenes used in the in situ synthesis/assay.



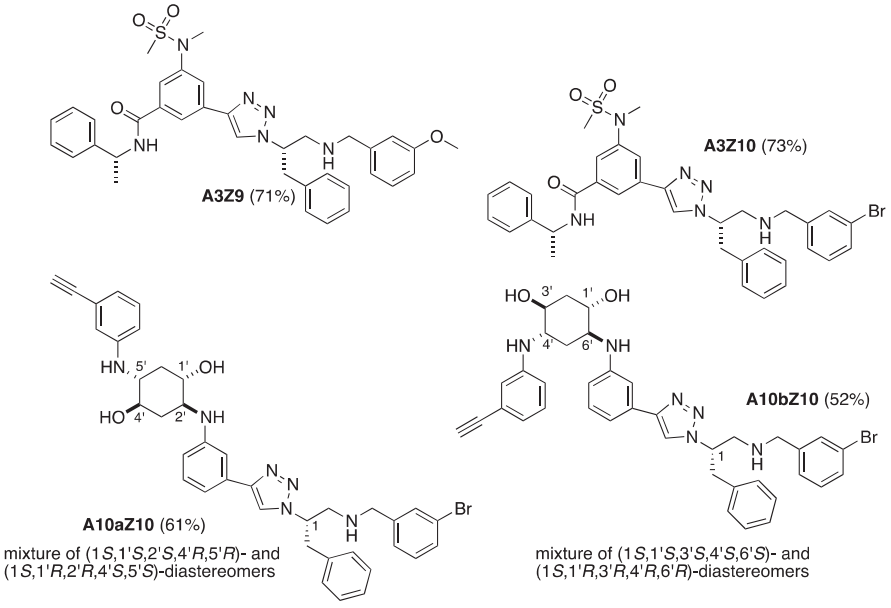
Scheme 1. Synthesis of the azide components. Reagents and conditions: (a) $(COCl)_2$, DMSO, DIPEA, CH_2Cl_2 −78 °C to rt; (b) Ala-$NHR^{2'}$or 3-$R^{2'}$-$C_6H_4CH_2NH_2$, $MgSO_4$, $NaBH(OAc)_3$, $(CH_2Cl)_2$; (c) Pd/C, H_2, CH_3OH; (d) TfN_3, $CuSO_4$, $H_2O/CH_3OH/CH_2Cl_2$, 1:3:1; (e) 4 N HCl in 1,4-dioxane.

To assess why the triazole-linked reduced amide inhibitors exhibited such low inhibition potency, **A3Z10** and potent oxadiazole-linked tertiary carbinamine **2a** were docked within the active site of the BACE1 enzyme. Ligands were removed from five published BACE1-inhibitor co-crystal structures (PDB ID: 2PH6,[19] 2IRZ,[8] 2B8L,[20] 2IS0,[8] and 2QZL[5]) and the resulting PDB objects were converted into ICM (internal coordinate mechanics) objects;[21,22] these represent five alternative conformations of BACE1. The best docking poses of **2a** and **A3Z10** within the active sites of these five structures were then found through in silico docking using the ICM docking module.[22,23] The most favorable docking scores were found for the 2PH6-derived structure, and Figure 4 shows both **2a** and **A3Z10** overlaid in the active site of this structure of BACE1. From Figure 4 it is apparent that the left-hand portion of both ligands adopt a very similar docking pose. However, one can clearly see that the ammonium (N1) of **A3Z10** is no longer in an optimal position in which to form a salt bridge to the D228 carboxylate, which is assumed to be a necessary feature of potent reduced amide BACE1 inhibitors.[5] With regard to the significant loss of potency of triazole-linked tertiary carbinamine **2b** relative to **2a**, our docking studies show very similar docking poses of these compounds, but with a significantly reduced docking score for **2b**. Rajapakse et al. have attributed the disparate affinities of **2a** and **2b** to differences in conformational constraints provided by the oxadiazole and triazole rings.[11] Thus it appears that significant re-design of our triazole-linked reduced amide isosteres would be necessary to achieve potent inhibition. It is possible that the triazole, however oriented, is not a good P_2 amide surrogate for this subclass of BACE1 inhibitors.

Since the P_3–P_2 region of **A10b-** and **A10a**-derived triazole inhibitors differs significantly from that of **A3Z10**, **1**, and **2a**, we also performed docking studies of the diastereomers of **A10bZ10** with BACE1. Although neither diastereomer docked correctly in the 2PH6-derived structure, acceptable docking poses for both the (1*S*,1′*S*,3′*S*,4′*S*,6′*S*)- and (1*S*,1′*R*,3′*R*,4′*R*,6′*R*)-diastereomers were obtained with the 2IRZ-derived structure. It thus seems possible that the micromolar inhibition afforded by **A10bZ10** could be attributed to active site occupancy.

In summary, 120 reduced amide isosteres were screened using a high throughput in situ synthesis/screening protocol. Of these, 4

Table 1
IC_{50} values for the most potent triazole-linked reduced amide inhibitors and control HEA inhibitor 4 (synthesized yield in parenthesis)



Compound	IC_{50} (μM)	95% CI (μM)
A3Z9	7.0	5.7–8.6
A3Z10	2.0	1.9–2.1
A10aZ10	3.4	3.2–3.7
A10bZ10	2.4	2.2–2.7
4 (Fig. 1)	0.020	0.019–0.021

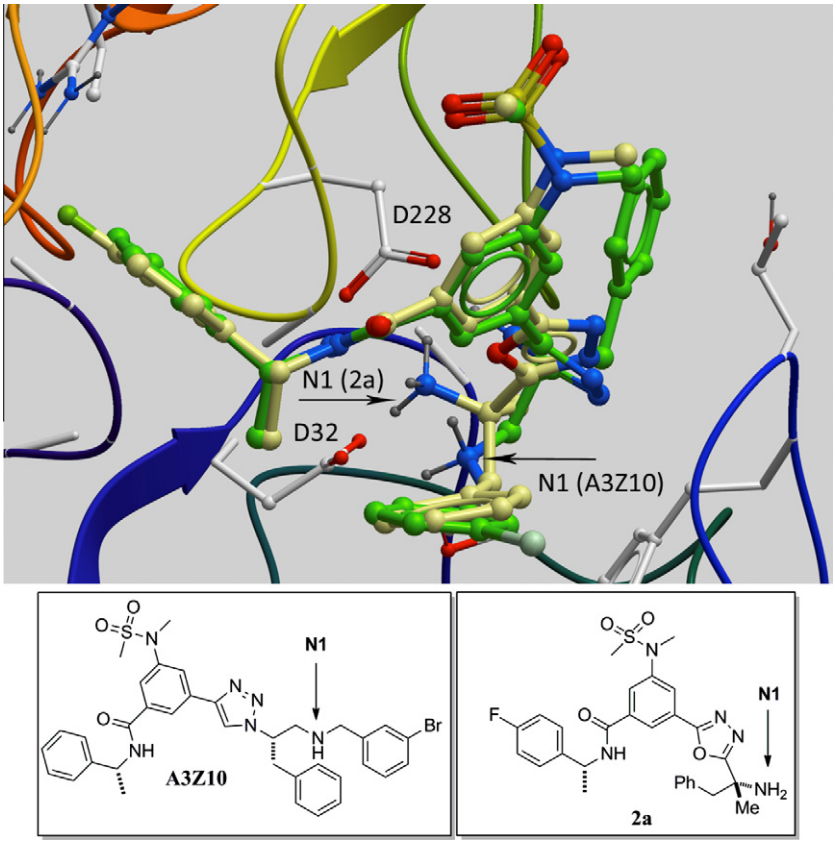


Figure 4. In silico docking of **2a** (off-white) and A3Z10 (green).

compounds (**A3Z9**, **A3Z10**, **A10aZ10**, **A10bZ10**) evidenced significant inhibition at 10 μM in a cell-free FRET assay, and were further examined, revealing single-digit micromolar IC_{50} values. Additionally, we have shown that the copper-mediated diazotransfer to primary amines can be performed in the presence of unprotected secondary amines. We believe that the synthetic strategy demonstrated herein will be useful for preparation of functionalized azides for application in 'click' chemistry-based drug discovery of reduced amide inhibitors for other proteases.

Acknowledgments

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Supplementary data

Supplementary data (synthetic procedures, analytical data, and details of the BACE1 inhibition assay procedure) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.007.

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