Identification of a Small Molecule Nonpeptide Active Site β-Secretase Inhibitor That Displays a Nontraditional Binding Mode for Aspartyl Proteases

Craig A. Coburn,*,‡ Shawn J. Stachel,‡ Yue-Ming Li,† Diane M. Rush,‡ Thomas G. Steele,‡ Elizabeth Chen-Dodson,‡ M. Katharine Holloway,‡ Min Xu,‡ Qian Huang,‡ Ming-Tain Lai,‡ Jillian DiMuzio,‡ Ming-Chih Crouthamel,‡ Xiao-Ping Shi,‡ Vinod Sardana,‡ Zhongguo Chen,‡ Sanjeev Munshi,‡ Lawrence Kuo,‡ Gergely M. Makara,§ D. Allen Annis,§ Praveen K. Tadikonda,§ Huw M. Nash,§ Joseph P. Vacca,‡ and Tong Wang§

Departments of Medicinal Chemistry, Biological Chemistry, Structural Biology and Molecular Systems, Merck Research Laboratories, West Point, Pennsylvania 19486-0004, and Department of Chemistry, NeoGenesis Pharmaceuticals, 840 Memorial Drive, Cambridge, Massachusetts 02139

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Abstract: A small molecule nonpeptide inhibitor of β-secretase has been developed, and its binding has been defined through crystallographic determination of the enzyme−inhibitor complex. The molecule is shown to bind to the catalytic aspartate residues in an unprecedented manner in the field of aspartyl protease inhibition. Additionally, the complex reveals a heretofore unknown S_3 subpocket that is created by the inhibitor. This structure has served an important role in the design of newer β-secretase inhibitors.

Over the past five years there has been considerable progress in understanding the etiology of Alzheimer's disease (AD). A key advance was the identification of the enzyme, β-secretase (Asp-2, BACE-1), that plays a critical role in the amyloid cascade.[1] BACE-1 is a unique member of the pepsin family of aspartyl proteases that initiates the production of the amyloidogenic Aβ peptide, the principle component of the senile plaques found in postmortem analysis of AD patients. Furthermore, recent reports have demonstrated a direct correlation between increased β-secretase activity and Aβ production in AD brain tissue.[2] Genetic deletion of β-secretase in mice has also been shown to prevent Aβ production and subsequent Aβ related events.[3] Since these knockout mice are otherwise healthy, inhibition of BACE-1 has emerged as an attractive therapeutic target for the treatment of AD.

Efforts to achieve pharmacological inhibition of aspartyl proteases have a long history driven by interest in HIV and renin programs.[4] Most of the successful inhibitors have evolved by truncation of a substrate-based polypeptide and concomitant replacement of the cleavable amide bond by a noncleavable transition state isostere. Such replacements for the scissile amide bond mimic the putative enzymatic reaction intermediate and result in potent inhibition. To date, a number of β-secretase inhibitors based on this design principle have been published and several have been cocrystallized with a truncated version of BACE-1.[5] However, β-secretase inhibitors based on this strategy must ultimately overcome the historical problems associated with peptidelike structures such as low oral bioavailability, poor blood−brain barrier permeability, and susceptibility to P-glycoprotein transport.[6]

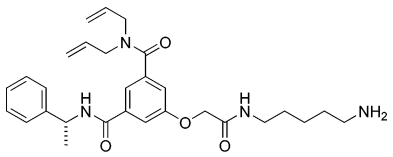

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Figure 1. Nonpeptide BACE-1 inhibitor **1**.

Weary of the difficulties associated with advancing a substrate-based lead, we chose to focus on the identification of a nonpeptide inhibitor from the outset of the program. Our efforts to identify such inhibitors hinged on the ability to rapidly assess potential inhibitors at high enzyme concentrations. Toward this end, we applied the Automated Ligand Identification System (ALIS) technology developed by scientists at NeoGenesis as a tool for lead generation.[7] The high throughput screening campaign involved incubation of BACE-1 with mass-encoded compound libraries followed by automated microscale size exclusion chromatography. Compounds that bound to BACE-1 were dissociated from the enzyme during subsequent reverse phase HPLC, analyzed by mass spectroscopy, and identified according to the appropriate mass information embedded in each library. *Through application of this strategy, a single 1,3,5-trisubstituted aromatic structure emerged from the multimillion compound library!* This structure was identified as the aminopentyl oxyacetamide (**1**), a compound that reproducibly inhibited β-secretase in five different enzymatic assays at IC_{50} values equal to 25 μM.[8] Selective inhibition of BACE-1 compared to related mammalian aspartyl proteases (BACE-2, renin, cathepsin D) was also observed. This small molecule (MW = 506), nonpeptide inhibitor was found to be a reversible inhibitor of BACE-1 and was competitive with the known inhibitor, Stat-Val (KTEEISEVN (statine)-VAEF).[9] All of these data taken together gave us confidence that compound **1** was indeed a novel active site inhibitor of β-secretase.

Efforts aimed at improving the potency of the lead compound focused on each of the three subunits of the benzenoid core. Subtle modifications to the length as well as the atomic composition of the oxyacetamide side chain all resulted in loss of activity. SAR at the tertiary amide site revealed that several amide surrogates, such as the 2-cyanophenyl group (**2**) or the sulfonate ester (**3**), could be employed to increase the potency of the inhibitor (Table 1). The addition of a fluorine atom at the 4-position of the α-methylbenzamide served to increase potency an additional 2-fold when compared to **3**. Also interesting was the requirement for the

* Address correspondence to Craig A. Coburn, Tel.: (215)-652-5511, E-mail: craig_coburn@merck.com.
† Present address: Sloan-Kettering Cancer Center, Box 459, 1275 York Ave., New York, NY 10021.
‡ Merck & Co., Inc.
§ NeoGenesis.

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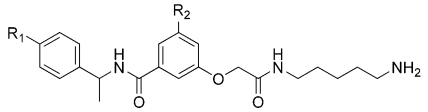
Table 1. SAR of BACE-1 Inhibitors



compound	stereochem	R_1	R_2	BACE-1 IC_{50} (μM)
1	*R*	H	C(O)N(allyl)$_2$	25 ± 2.2
2	*R*	H	2-CN-Ph	21 ± 1.5
3	*R*	H	OSO_2CH_2Ph	3.4 ± 0.42
4	*S*	H	OSO_2CH_2Ph	>100
5	*R*	F	OSO_2CH_2Ph	1.4 ± 0.28

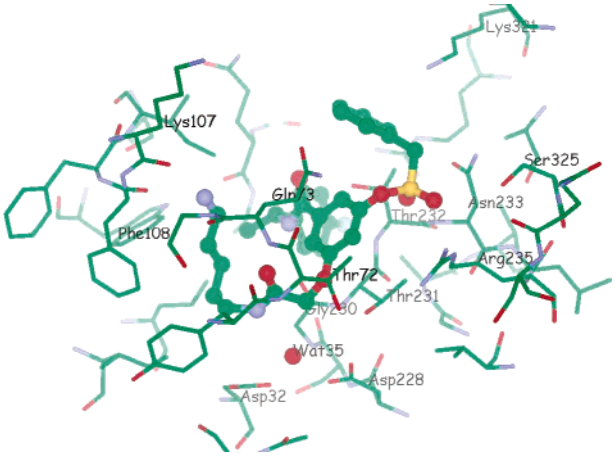


Figure 2. X-ray crystal (1.8 Å) structure of oxyacetamide **5** in the BACE-1 active site.

R-stereochemistry at the chiral α-methyl group to maintain good enzymatic activity. Compound **5** displayed an IC_{50} versus BACE-1 of 1.4 μM ($n = 3$) in an electrochemilluminescence (ECL) enzyme inhibition assay. Comparative inhibition against related aspartyl proteases was negligible (Cat D, renin > 500 μM) and the IC_{50} against BACE-2 was 137 μM.

Using a human BACE-1 variant expressed in *E. coli*, we were able to obtain a single crystal of the BACE-1/inhibitor **5** complex at a resolution of 1.8 Å. To enable crystallization in a highly ordered lattice, a construct that contained two mutations, K75A and E77A, was employed. The kinetic and binding properties of this mutant were unchanged from those of the wild type similarly expressed and purified.[10] Details of our X-ray crystallographic studies on both the apo and bound forms of human BACE-1 will be given separately.

Examination of the enzyme/inhibitor complex revealed that compound **5** occupies the S_4 to S_1 subsites of BACE-1 and does not have any interaction with the 'prime-side' of the enzyme. Most notably, compound **5** does not show any direct contact with the catalytic aspartic acids, Asp32 and Asp228 (Figure 2). Instead, inhibitor **5** forms a hydrogen bond from the oxyacetamide NH to a water molecule situated between the aspartyl dyad (see Figure 3) that is analogous to the catalytic water observed in the apo crystal structures of other aspartyl proteases, e.g. renin,[11] cathepsin D,[12] and endothiapepsin.[13] *With the exception of a recently reported Ser-Thr dipeptide ligand for endothiapepsin during a 12-year crystallization period, this water-mediated binding mode is unique among aspartyl protease inhibitors.*[14] Regarding the stereochemistry of the α-methyl-benzamide ligand, analysis of the crystal

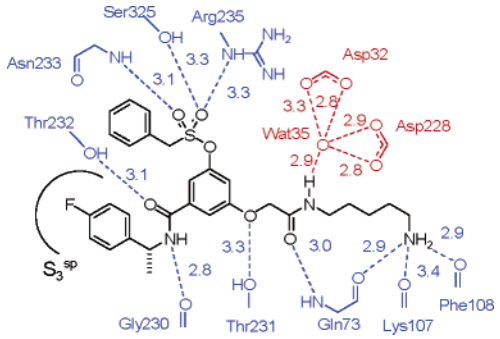


Figure 3. Hydrogen bonding network for BACE-1/inhibitor **5** complex.

structure revealed that the P_3 α-methyl group packs firmly against Ile110 while orienting the *p*-fluorophenyl ring toward S_3 thus creating a novel S_3 subpocket (S_3^{sp}). This finding is unexpected for BACE-1, as small hydrophobic groups are normally preferred at S_3 in the natural substrate (valine) and previously reported substrate-based inhibitors.[5] This phenomena, however, is not completely unprecedented and is observed in the binding of aliskiren to renin.[15] Other notable features of this unique structure include: (1) coiling of the terminal aminopentyl chain back into the S_1 pocket, anchored by hydrogen bonds to the backbone carbonyl oxygens of Gln73, Lys107, and Phe108; (2) weak hydrogen bonding between one of the sulfonate oxygens and Arg235, a residue unique to BACE-1 and BACE-2; and (3) a cation−π interaction between Lys321 and the aromatic face of the benzylsulfonate substituent.

Our high throughput screening effort has resulted in the identification of a low micromolar nonpeptide inhibitor of BACE-1. The unprecedented binding mode of this novel inhibitor coupled with the structural information gleaned therein has guided our drug design effort toward more potent BACE-1 inhibition.

Note Added after ASAP Posting. An additional coauthor, Tong Wang, was added to the author byline in the version of the manuscript posted October 30, 2004. The new version was posted November 5, 2004.

Supporting Information Available: Experimental procedure for the synthesis of compound **5**. PDB file for BACE-1/inhibitor **5** complex has been deposited with the Protein Data Bank (PDB identifier 1TQF). This material is available free of charge via the Internet at http://pubs.acs.org.

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