Supporting information for

The Discovery of AM-6494: A Potent and Orally Efficacious β-Site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE1) Inhibitor with *in vivo* Selectivity over BACE2

Liping H. Pettus,*[1] Matthew P. Bourbeau,[1] Jodi Bradley,[2] Michael D. Bartberger,[3] Kui Chen,[4] Dean Hickman,[5] Michael Johnson,[6] Qingyian Liu,[1] James R. Manning,[1] Adrian Nanez,[7] Aaron C. Siegmund,[1] Paul H. Wen,[2] Douglas A. Whittington,[3] Jennifer R. Allen,[1] and Stephen Wood[2]

[1]Medicinal Chemistry, [2]Neuroscience Research, [3]Molecular Engineering, [4]Discovery Technologies, [5]Pharmacokinetics and Drug Metabolism, [6]Research Automation and Technologies, [7]Comparative Biology and Safety Studies, Amgen Discovery Research, Thousand Oaks, CA 91320

Table of Contents

Table SI-1. Cross species biochemical IC_{50} data for compounds **20** and **25**

Compd	human BACE1 IC_{50} (µM)	rat BACE1 IC_{50} (µM)	mouse BACE1 IC_{50} (µM)	dog BACE1 IC_{50} (µM)	monkey BACE1 IC50 (µM)
20	4.3271E-4 +/- 1.77E-4 (n=17)	6.1775E-4 +/- 5.25E-4 (n=4)	3.5975E-4 +/- 8.54E-5 (n=4)	4.205E-4 +/- 4.66E-5 (n=4)	3.4658E-4 +/- 1.11E-4 (n=4)
25	2.0722E-4 +/- 8.28E-5 (n=18)	3.0675E-4 +/- 1.72E-4 (n=4)	1.9525E-4 +/- 1.0E-4 (n=4)	1.73E-4 +/- 7.37E-5 (n=4)	1.5737E-4 +/- 5.16E-5 (n=4)

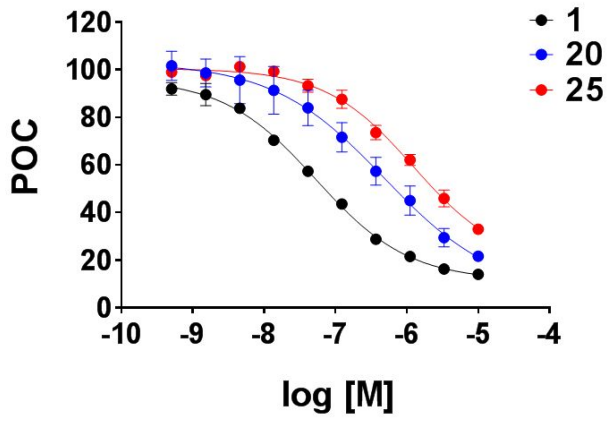

Figure SI-1. Dose response curves of **1**, **20**, and **25** tested in BACE2 cell-based (HEK293 cells) assay plotted as percent of control (POC). IC_{50} values are 53.4, 484.5, and 1240 nM for **1**, **20**, and **25**, respectively.

Experimental procedure for 20 (AM-6494) in complex with BACE1 (PDB 6PZ4)

The extracellular domain of BACE1 was expressed, purified, and crystallized according to published procedures.[1] Inhibitor-bound BACE1 crystals were prepared by soaking apo crystals in a mother liquor solution supplemented with 1 mM of compound **20** (AM-6494) for 5 h at room temperature. Crystals were transferred briefly into a cryo solution consisting of 25% (w/v) PEG 5000 MME, 0.1 M sodium citrate (pH 6.6), 0.2 M ammonium iodide, and 20% (v/v) glycerol prior to being flash frozen in liquid nitrogen. Diffraction data were collected on the APS SER-CAT 22-BM equipped with a Mar 225 detector. Images were processed using the HKL suite of programs.[2] The structures were refined using REFMAC,[3] and model building was performed with COOT.[4]

References

(1) Patel, S.; Vuillard, L.; Cleasby, A.; Murray, C.M.; Yon, J. Apo and inhibitor complex structures of BACE (β-secretase) *J. Mol. Biol.* **2004**, *343*, 407-416.

(2) Otwinowski, Z.; Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **1997**, *276*, 307-326.

(3) Murshudov, G.N.; Vagin, A.A.; Dodson, E.J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr.* **1997**, *D53*, 240-255.

(4) Emsley, P.; Cowtan, K. *Coot*: model-building tools for molecular graphics. *Acta Crystallogr*. **2004**, *D60*, 2126-2132.