Supporting information for…

A Highly Potent and Selective Caspase 1 Inhibitor that Utilizes a Key 3-Cyanopropanoic Acid Moiety.

Matthew B. Boxer, Amy M. Quinn, Min Shen, Ajit Jadhav, William Leister, Anton Simeonov, Douglas S. Auld and Craig J. Thomas*

NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, 9800 Medical Center Drive, Rockville, Maryland 20850, USA.

Send proofs to:
Dr. Craig J. Thomas
NIH Chemical Genomics Center
NHGRI, National Institutes of Health
9800 Medical Center Drive
Building B, Room 3005
MSC: 3370
Bethesda, MD 20892-3370
301-217-4079
301-217-5736 (fax)
craigt@mail.nih.gov

NCGC00183434 Ki Determination

Active caspase-1 was purchased from Enzo Life Sciences (Plymouth Meeting, PA) as recombinant enzyme expressed in *E.coli* from a construct encoding residues 120-404, with a D381E substitution to resist autoproteolysis. The inhibitor constant, K_I, was determined for NCGC001183434 (**4**) in reactions of 2 nM caspase-1 with varied concentrations of Ac-LEHD-AMC substrate. A series of six NCGC00183434 (**4**) dilutions (final 8 pM – 8 nM) in DMSO were prepared in duplicate in 384-well matrix plates, and 2 μL of compound were transferred to 30 μL caspase-1 in 384-well black assay plates with a CyBi-Well (CyBio Inc., Woburn, MA) 384-channel simultaneous pipettor. Following a 15 min coincubation of enzyme with NCGC00183434 (**4**), reactions were initiated with the CyBi-Well transfer of 10 μL Ac-LEHD-AMC (final 0.5 – 50 μM) dilutions in buffer from a 384-well source plate to the assay plate. The change in AMC fluorescence was measured over 20 min, and data were normalized to no-enzyme and DMSO controls. Global curve-fitting of data was performed with GraFit 5 software. A competitive inhibition model was fitted to the data which estimated a K_I=0.4 nM and a K_M for the AMC substrate of 15 μM