Structural Requirements for the Inhibition of Calcium Mobilization and Mast Cell Activation by the Pyrazole Derivative BTP2

Mankit Law[1,2,4], J. Luis Morales[1], Laurie F. Mottram[3], Archana Iyer[1,2,#], Blake R. Peterson[3,†], and Avery August[1,2,4,*]

[1]Center for Molecular Immunology and Infectious Disease, Department of Veterinary and Biomedical Sciences, [2]Huck Institutes of the Life Sciences, Immunology and Infectious Diseases Graduate Program, [3]Department of Chemistry, The Pennsylvania State University, University Park, PA 16802, USA, [4]Department of Microbiology & Immunology, Cornell University, Ithaca, NY, USA.

Running title: SAR of BTP for mast cell degranulation

[#]Present Address: Joint Centers for Systems Biology, Columbia University, 1130 St. Nicholas Avenue, New York, NY 10032, USA.

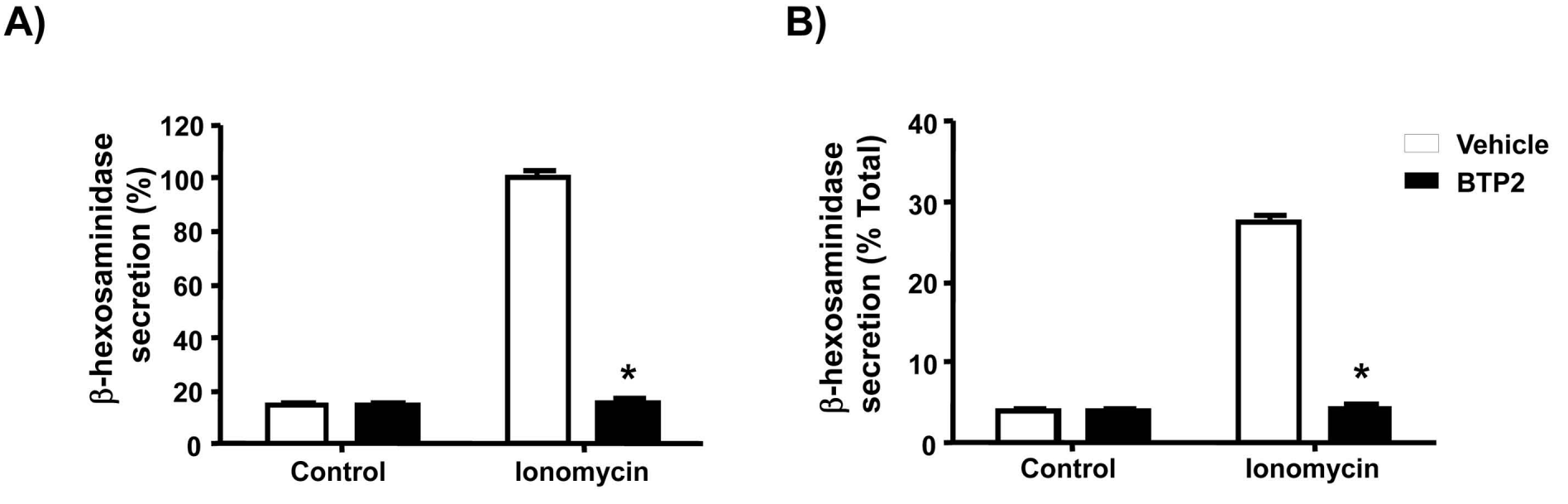
[††]Present Address: Department of Medicinal Chemistry, University of Kansas, 1251 Wescoe Hall Drive, 4070 Malott Hall, Lawrence, Kansas 66045-7582, USA

[*]Correspondence at current address: Avery August, Department of Microbiology & Immunology, College of Veterinary Medicine, VMC 5117, Cornell University, Ithaca, NY, 14853-6401, USA. (607) 253-3401 (Ofc), (607) 253-4058 (Fax), email: averyaugust@cornell.edu

Supplemental Material

Figure Legend

Supplementary Figure 1. BTP2 inhibits mast cell degranulation and release of β-hexosaminidase *in vitro*. RBL-2H3 cells were stimulated for 1 hour with ionomycin (500 nM) following pre-treatment with BTP2 (1 μM) or vehicle (DMSO) for 1 hr. After stimulation, cells were lysed with 1% Triton-X, and the extracts were analyzed for their β-hexosaminidase activities (total - test). β-hexosaminidase activity in unstimulated cells (spontaneous) was subtracted from the enzyme activity from stimulated cells (test). (**A**) Data expressed as a percent of ionomycin induced release. (**B**) Data expressed as a percentage of total β-hexosaminidase released into the supernatant. This was calculated using the following formula: release (% of total) = ((test - spontaneous)/(total – spontaneous)) X 100. Data are expressed as the mean ± SEM of 3 independent experiments. ***, $P < 0.001$ vs. Vehicle.



Supplemental Figure 1