Supporting Information

Design, Synthesis, Docking, and Biological Evaluation of Novel Diazide-containing Isoxazole- and Pyrazole-based Histone Deacetylase Probes

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In-house HDAC1 and HDAC2assay protocols: In this assay, recombinant, full-length HDAC1 or HDAC2 (BPS Bioscience, San Diego, CA) was diluted to 40 ng (for HDAC1) or 50 ng (for HDAC2) with HDAC assay buffer (25 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/mL BSA) and pre-incubated with 30 µL of inhibitor for 5 min, then 10 µL, 125 µM of HDAC fluorophore-conjugated substrateBoc-L-Lys (Ac)-AMC (Chem-Impex, Wood Dale, IL) was added and the mixture incubated for 35 min (for HDAC1)/ 60 min (for HDAC2) at room temperature. The reaction was quenched with HDAC assay developer (1 mg/mL trypsin in 25 mM Tris-HCl, pH 8.0, 137 mM NaCl, 5 µM TSA, 2.7 mM KCl, 1 mM $MgCl_2$) for 30 min. The plate was read on an OptimaPolar Starmicroplate reader (BMG labtech) at excitation wavelength 360 nm and emission wavelength 460 nm. The IC_{50} values were determined using the GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA).

Activity assays for HDACs 4–7 and 9–11: These assays were performed by the Reaction Biology Corporation, Malvern, PA, using the Reaction Biology HDAC Spectrum platform, web-site www.reactionbiology.com.

The HDAC4, 5, 6, 7, 8, 9, 10, and 11 assays used isolated recombinant human proteins and a general substrate- fluorogenic peptide from p53 residues 379-382 (RHKKAc). Compounds were dissolved in DMSO and tested in a 10-dose IC_{50} mode with 3-fold serial dilution starting at 100 µM. Control compound Trichostatin A (TSA) was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 10 µM. IC_{50} values were extracted by curve-fitting the dose/response slopes.

Activity assays for matrix metallo-proteases (MMPs)-1, 3, 9, 14: These assays were performed by the Reaction Biology Corporation, Malvern, PA, web-site www.reactionbiology.com.

Compounds were dissolved in DMSO and tested in a 10-dose IC_{50} mode with 3-fold serial dilution starting at 100 µM. Control compound galardin (GM6001)[1] was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 100 nM. The protease activities were monitored as a time-course measurement of the increase in fluorescence signal from fluorescently-labeled peptide substrate, and initial linear portion of slope (signal/min) was analyzed. IC_{50}values were extracted by curve-fitting the dose/response slopes.

References:

1. Santiskulvong, C., Rozengurt, E. Galardin (GM6001), a broad-spectrum matrix metalloproteinase inhibitor, blocks bombesin- and LPA-induced EGF receptor transactivation and DNA synthesis in rat-1 cells. *Exp. Cell Res.* **2003**, 290, 437–446.