Supplementary Material

Synthesis of thiazolyl-based hydroxamic acids as histone deacetylase inhibitors

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Docking studies

The structures of the ligands were prepared using the software ChemDraw 16.0 and Discovery studio and used as input for AutoDock tools version1.5.7 (ADT). The crystal structures of the proteins HDAC2 (PDB:5IWG)[1], HDAC4 (PDB: 5A2S)[2], HDAC6 (PDB: 5EDU)[3] and HDAC8 (PDB: 5FCW)[4] were downloaded from the Protein Data Base. All waters molecules, buffer and non-interacting ions were removed with Autodock 4.2. The cleaned protein structures were used for the grid box generation. Using Autodock 4.2, a Lamarkian genetic algorithm was applied and the search parameters were set to 100 GA runs for each ligand with a population size of 150, maximum number of $2.5 \cdot 10^6$ energy evaluations, a maximum number of $2.7 \cdot 10^4$ generations, a mutation rate of 0.2 and a crossover rate of 0.8 and the default dockings parameters. Populations of 100 docking poses were generated for each run and organized in clusters and the first pose of the cluster demonstrating coordinative interactions between the hydroxamic acid and the zinc ion (distance <3.5 Å) was selected.

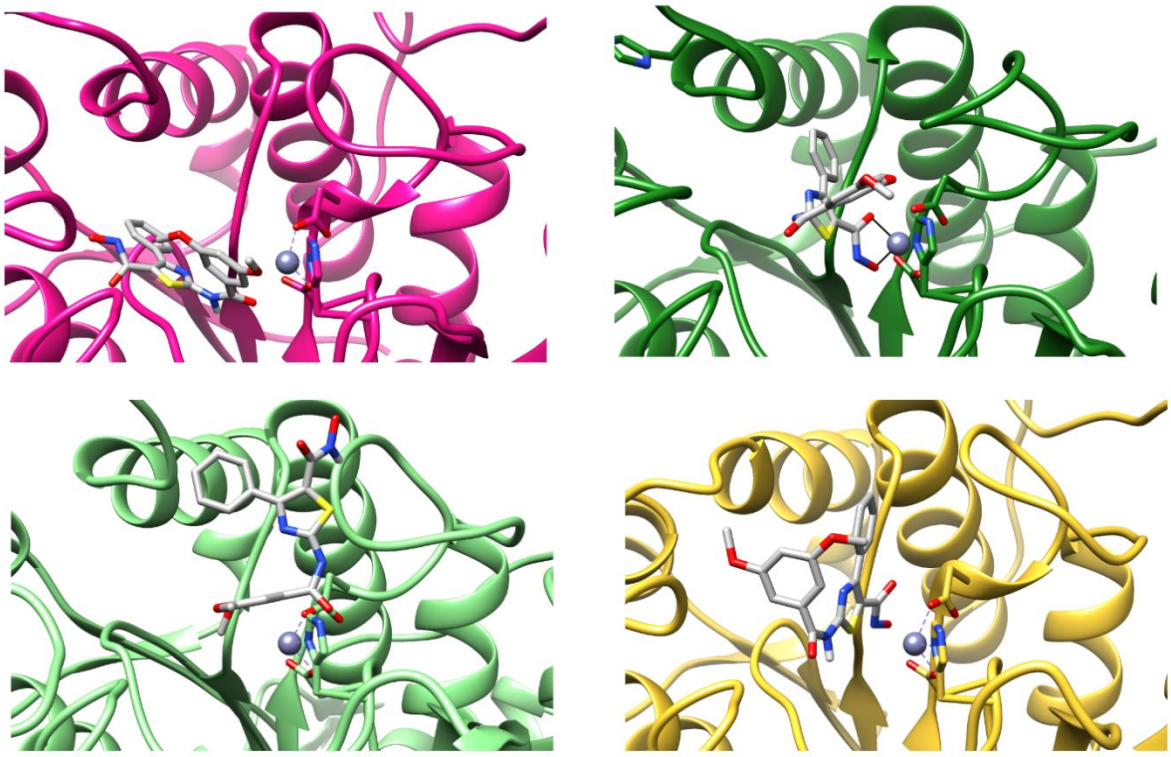

Figure 1. A) Docking of **9a** in the crystal structure of HDAC2 B) Docking of **9a** in the crystal structure of HDAC4 C) Docking of **9a** in the crystal structure of HDAC6 D) Docking of **9a** in the crystal structure of HDAC8.

Biological evaluation

Reagents

Cisplatin was purchased from Sigma (Munich, Germany) and dissolved in 0.9% sodium chloride solution. Stock solutions (10 mM) of vorinostat (Selleckchem, Houston, Texas, USA), Panobinostat, CHDI-00390576-000-004 (kindly provided by the CHDI Foundation Inc., New York, USA) and the respective compounds were prepared with DMSO and diluted to the desired concentrations with the appropriate medium.

Cell lines and cell culture

The human peripheral blood monocytic cell line THP-1 was kindly provided by Prof. Dr Hanenberg (Heinrich-Heine-University, Duesseldorf, Germany). It was cultured in RPMI 1640 containing 10 % heat inactivated fetal calf serum 120 IU/mL penicillin and 120 µg/mL streptomycin at 37 °C in a humidified atmosphere of 5 % CO_2.

MTT Cell Viability Assay

MTT assay was performed as previously described. [5–7] Cells were seeded at a density of 15 000 cells/well in 96-well plates (Corning, Kaiserslautern, Germany) in 90 µl culture medium. After 24h preincubation, cells were incubated with the test compounds. After 72 h, MTT solution (5 mg/mL in phosphate buffered saline) was added. The precipitate was dissolved in acidic isopropanol solution (165 µL concentrated HCl (VWR, Langenfeld, Germany) in 50 mL isopropanol (VWR, Langenfeld, Germany). Absorbance was measured at 595 nm and 690 nm in a NOVOstar microplate-reader (BMG LabTech, Offenburg, Germany).

Whole-Cell HDAC Inhibition Assay

The cellular HDAC assay is based on an assay published by Heltweg and Jung[8], Ciossek et al.[9], and Bonfils et al.[10] with minor modifications as described in Marek et al.[5]. Cells were seeded at a density of 50 000 cells/well in 96-well plates (Corning, Kaiserslautern, Germany) in 90 µl of culture medium. After 24 h, cells were incubated for 18 h with the test compounds. 10 µl of 3 mM HDAC substrate in DMSO (VWR, Langenfeld, Germany) of either Boc-Lys(ε-Ac)-AMC (Bachem, Bubendorf, Switzerland) or Boc-Lys(ε-TFAc)-AMC (Bachem, Bubendorf, Switzerland) was incubated for 3h under cell culture conditions. 100 µl/well of the stop solution (25 mM Tris-HCl (pH 8), 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1% NP40, 2.0 mg/mL trypsin and 10 µM vorinostat for Boc-Lys(ε-Ac)-AMC and 10 µM panobinostat for Boc-Lys(ε-TfAc)-AMC) was added and incubated for 3 h. Fluorescence intensity was measured at an excitation of 320 nm and emission of 520 nm in a NOVOstar microplate reader (BMG LabTech, Offenburg, Germany).

Enzyme HDAC Inhibition Assay

All human recombinant enzymes were purchased from Reaction Biology Corp. (Malvern, PA, USA). The HDAC activity assays for HDAC2 (cat nr. KDA-21-277), HDAC4 (cat nr. KDA-21-279), HDAC6 (cat nr. KDA-21-213), and HDAC8 (cat nr. KDA-21-481) were performed in 96-well-plates (Corning, Kaiserslautern, Germany). Briefly, 20 ng of HDAC2/8, 17.5 ng of HDAC6 and 2 ng of HDAC4 per reaction were used. Recombinant enzymes were diluted in assay buffer (50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, and 1 mg/mL BSA). After a 5 min incubation step the reaction was started with 10 µL of 300 µM (HDAC2), 150 µM (HDAC6) Boc-Lys(ε-Ac)-AMC (Bachem, Bubendorf, Switzerland) or 100 µM (HDAC4), 60 µM (HDAC8) Boc-Lys-(ε-TFAc)-AMC (Bachem, Bubendorf, Switzerland). The reaction was stopped after 90 min by adding 100 µL stop solution (16 mg/mL

trypsin, 2 µM vorinostat for HDAC2/6/8, 4 µM CHDI-00390576-000-004 for HDAC4 in 50 mM Tris-HCl, pH 8.0, and 100 mM NaCl. 15 min after the addition of the stop solution the fluorescence intensity was measured at excitation of 355 nm and emission of 460 nm in a NOVOstar microplate reader (BMG LabTech, Offenburg, Germany).

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