Supporting Information

Structural Basis of the Antiproliferative Activity of Largazole, a Depsipeptide Inhibitor of the Histone Deacetylases

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Experimental Section

Inhibitor preparation. S-trityl-protected largazole was prepared according to published procedures[27]. This material (10 mg, 20 μmoles) was dissolved in dry dichloromethane (3 mL) and cooled on ice[17]. Triisopropylsilane (11 μL, 8.5 mmoles) and trifluoroacetic acid (134 μL, 200 mmoles) were added, and the reaction mixture was stirred on ice for ~2.5 hours and then allowed to reach room temperature. The reaction mixture was concentrated and purified using column chromatography (silica gel with a solvent mixture of 100% ethyl acetate). The fractions containing the deprotected thiol were combined and solvent was removed under vacuum to afford the product as a clear oil (5 mg, 50% yield). The final product was dissolved in dimethyl sulfoxide to a final concentration of 40 mM and stored at -80 °C.

Structure Determination and Refinement. Wild-type HDAC8 was recombinantly expressed in BL21(DE3) *E. coli* cells and purified according to published procedures[21], modified such that only affinity and size exclusion chromatographies were used for protein purification. Crystals of HDAC8 complexed with the largazole thiol were obtained in 1-2 days using the hanging drop vapor diffusion method. Briefly, a 2 μL hanging drop of protein solution (~5 mg/mL HDAC8, 50 mM Tris (pH 8.0), 150 mM KCl, 5% glycerol (vol/vol), 1 mM dithiothreitol, 30 mM Gly-Gly-Gly, 2 mM largazole thiol) was mixed with a 2 μL drop of precipitant solution (0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES) (pH 5.3), 4 mM tris(2-carboxyethyl)phosphine (TCEP), 1-6% polyethylene glycol (PEG) 35,000), and equilibrated against a 600 μL reservoir of precipitant solution at 21 ºC. Crystals were harvested and flash-cooled in precipitant buffer supplemented with 20% PEG 35,000 and 20% glycerol. Crystals diffracted X-rays to 2.14 Å resolution at the National Synchrotron Light Source, beamline X29

(Brookhaven National Laboratory). Data were collected using incident radiation with λ = 0.9795 Å at 100 K. Diffraction data were indexed and scaled using HKL2000[28]. Crystals belonged to space group $P2_1$ with unit cell dimensions a = 54.06 Å, b = 88.30 Å, c = 93.66 Å, β = 101.62º; with two monomers in the unit cell, the Matthews coefficient of 2.55 $Å^3$ / Da corresponds to a solvent content of 52%.

The structure was solved by molecular replacement[29] using the atomic coordinates of HDAC8 complexed with substrate (PDB code: 3EWF, less ions, solvent, and substrate) as a search probe in rotation and translation function calculations. Iterative cycles of refinement and model building were performed with CNS[30] or Phenix[31], and Coot[32], respectively, to improve the structure as monitored by R_{free}. Noncrystallographic symmetry restraints were used throughout refinement, with a final coordinate sigma value of 0.05. Atomic coordinates for the largazole thiol and solvent molecules were added during the final stages of refinement. Disordered segments in the final model include M1-S13, E85-I94, and I378-H389 in monomer A, and M1-Q12, Q84-S93, and E379-H389 in monomer B. The final Ramachandran plot indicated that 90.3% of the residues adopted most favored conformations, 9.4% were additionally allowed, 0.0% were generously allowed, and 0.3% were in disallowed conformations. Of the residues with disallowed conformations, L14 of monomer B is characterized by noisy electron density at the N-terminus, and Y100 in both monomers is characterized by well-defined electron density. Data collection and refinement statistics are recorded in Table S1.

References

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Table S1. Data collection and refinement statistics

	HDAC8-Largazole
Data collection	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	54.06, 88.30, 93.66
α, β, γ (°)	90.00, 101.62, 90.00
Resolution (Å)	2.14
R_{merge}	0.127(0.543)*
$I / \sigma I$	10.8(2.8)
Completeness (%)	98.8(98.3)
Redundancy	4.4(4.2)
Refinement	
Resolution (Å)	2.14
No. reflections	46833
R_{work} / R_{free}	0.204/ 0.245
No. atoms (per asym. unit)	
Protein	5553
Zinc	2
Potassium	4
Ligand	64
Water	504
Average *B*-factors (Å^2)	
Protein	27
Zinc	17
Potassium	21
Ligand	20
Water	34
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.1

* Values in parentheses are for highest-resolution shell.

Table S2. Enzyme-Inhibitor Interactions ≤ 3.2 Å[*]

HDAC8	*Largazole*	*Type of interaction*	*Distance (Å)*
Zn^{2+}	S_{21}	metal coordination	2.3
Y306 (OH)	S_{21}	hydrogen bond	3.2
D101 ($O_{\delta 2}$)	N_{14}	hydrogen bond	2.6
F208 ($C_{\delta 2}$)	C_{15}	van der Waals	3.1
water #6/#222[a,b]	O_1	hydrogen bond	2.9
water #223/#110[a]	O_{15}	hydrogen bond	2.8

*Distances are averaged between monomers A and B.

[a] Water molecule numbering in monomer A/B.

[b] Water #6/#222 (monomer A/B) also hydrogen bonds with the $N_{\varepsilon 2}$-H group of Zn^{2+} ligand H180.