**Identification of Novel HDAC8 selective inhibitors through ligand and structure based studies:** **Exploiting the Acetate Release Channel Differences Among Class I isoforms**

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**Table S1. Metrics of Phase Hypothesis obtained from Pharmacophore studies**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Survival** | **Survival -inactive** | **Post-hoc** | **Site** | **Vector** | **Volume** | **Selectivity** | **Activity** | **Inactive** |
| AADRR.2 | 3.382 | 1.817 | 3.382 | 1 | 1 | 0.387 | 1.386 | 7.638 | 1.565 |
| AAADR.13 | 2.499 | 0.869 | 2.499 | 0.3 | 0.9 | 0.294 | 1.268 | 7.538 | 1.63 |
| AAADR.14 | 2.499 | 1.223 | 2.499 | 0.3 | 0.9 | 0.294 | 1.325 | 7.638 | 1.276 |
| AAADR.18 | 2.499 | 1.223 | 2.499 | 0.3 | 0.9 | 0.294 | 1.325 | 7.638 | 1.276 |

Chart, bar chart

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**Figure S1:** Bar graphs showing the Enzyme inhibitory kinetics of compounds 1-3.

Diagram

Description automatically generated

**Figure S2:** Side chain placement of Trp 141 to accommodate inhibitor, in x-ray structures of HDAC8: 1T64 (blue) and 3SFF (green) shown in circle

**Diagram

Description automatically generated**

**Figure S3:** Overlay of binding poses of Compounds **1** (yellow), **2** (pink) and **3** (orange) within HDAC8 active site

|  |  |
| --- | --- |
| Chart, histogram  Description automatically generated | Chart  Description automatically generated |

**Figure S4:** Distance and Angle between Ser138 backbone O-atom and Trp 141 sidechain NH atoms

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**Figure S5:** Sequence Alignment of Class I HDAC isoforms. Red boxes show the variants in the AR Channel

**Characterization of Purchased Compounds**

A Bruker Ascend 400 MHz spectrometer was used to record 1H and 13C NMR spectra at room temperature (400 MHz for 1H and 100 MHz for 13C). Chemical shifts were recorded in ppm using TMS as internal standard and coupling constants were recorded in Hertz. Proton multiplicities were used to indicate as s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and m (many).

**Compound 1**

C15H15N3O3: 1H-NMR (400 MHz, DMSO-d6) δ ppm: 11.71 (1H, brs), 8.74 (1H, brs), 7.78 (1H, brs), 7.57-7.23 (8H, m). 5.94 (2H, brs), 4.92 (2H, brs). 13C-NMR (400 MHz, DMSO-d6), δ ppm: 165.2, 156.4, 141.3, 136.4, 133.4, 129.3 (2C), 129.1, 128.8 (2C), 121.1, 119.9, 117.0, 77.4. *m/z* 285.3 [M]+

**Compound 2**

C19H16N4O3S: 1H-NMR (400 MHz, DMSO-d6) δ ppm: 14.13 (1H, brs) 12.28 (1H, brs), 8.48 (1H, brs), 7.77 (1H, d, 8.0), 7.61 (1H, d, 8.4), 7.45-7.47 (3H, m), 7.43-7.31 (4H, m), 4.97 (2H, s), 4.85 (2H, s). 13C-NMR (400 MHz, DMSO-d6), δ ppm: 165.2, 157.3, 153.3, 148.6, 142.9, 136.2, 129.3 (2C), 128.8 (2C), 127.9, 127.4, 124.0, 122.6, 122.1, 77.8, 25.3. *m/z* 381.2 [M+H]+

**Compound 3**

C19H15N3O2S: 1H-NMR (400 MHz, DMSO-d6), δ ppm: 10.15 (1H, brs), 8.17 (1H, brs), 8.00 ((1H, brs), 7.62-7.53 (7H, m), 7.46 (3H, brs), 7.38 (2H, d, 6.0). 13C-NMR (400 MHz, DMSO-d6), δ ppm: 163.2, 162.8, 161.4, 152.9, 147.5, 137.9, 137.7, 136.5, 135.6, 132.5, 130.3, 130.2, 129.8, 129.3, 129.0, 128.8, 128.6, 128.3, 128.0. *m/z* 350.3 [M+H]+

**Compound 4**

C18H17N3O3: 1H-NMR (400 MHz, DMSO-d6) δ ppm: 7.45-7.52 (3H, m), 7.36-7.44 (3H, m), 5.02 (2H, s), 2.43 (3H, s), 2.22 (1H, m), 1.15 (2H, m), 1.05 (2H, m). 13C-NMR (400 MHz, DMSO-d6), δ ppm: 170.5, 161.5, 155.8, 138.6, 136.2, 129.4 (2C), 128.9, 128.8 (2C), 117.3, 107.5, 77.6, 17.7, 12.84, 12.31 (2C). *m/z* 323.3 [M]+

**Compound 5**

C25H22N2O4S: 1H-NMR (400 MHz, DMSO-d6), δ ppm: 10.54 (1H, s), 8.06 (1H, s), 7.90 (d, J=12, 3H), 7.78 (s, 1H), 7.67-7.40 (m, 10H), 3.81-3.75 (m, 1H), 3.67-3.62 (m, 1H), 3.57-3.51 (m, 1H), 2.78 (brs, 2H), 1.87-1.71 (m, 3H), 1.55-1.48) (m, 1H). 13C-NMR (100 MHz, DMSO-d6), δ ppm: δC  162.9, 141.6, 137.8, 135.2, 131.3, 130.2, 129.7, 129.2, 128.9, 128.3, 77.4, 67.7, 46.9, 28.7, 25.5. *m/z* 464.2 [M+H]+