**Supplementary Material**

**Discovery of selective inhibitors of the *Clostridium difficile* dehydroquinate dehydratase**

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|  |  |
| --- | --- |
| A |  |
| B |  |

**Supplementary Figure S1. Fit of initial rate measured under varying substrate and inhibitor concentrations to the mixed-type and competitive models.** (A)Fits for compound **1**. (B) Fits for compound **3**.

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| --- | --- | --- | --- |
| A |  | B |  |

**Supplementary Figure S2. NMR data for binding of compounds 1 and 2 to *cd*DHQD.** (A) Line broadening observed for compound **1** upon addition of *cd*DHQD or *se*DHQD. (B) Line broadening observed for compound **2** upon addition of *cd*DHQD or *se*DHQD. Note that line broadening was observed only in the presence of *cd*DHQD but not with *se*DHQD, demonstrating the selectivity of the compounds to *cd*DHQD. The experimental conditions were 10 μM DHQD, 100 μM compound in PBS/pH 7.2, 10% 2H2O at 25˚C. Data were acquired on a Bruker 900 MHz spectrometer equipped with a cryogenic triple resonance probe. Line widths were estimated using NMRDraw (Delaglio et al., 1995).



**Supplementary Figure S3. NMR data for binding of compound 3 to *cd*DHQD.** The experimental conditions were 10 μM DHQD, 100 μM compound **3** in PBS/pH 7.2, 10% 2H2O at 25˚C. Data were acquired on a Bruker 900 MHz spectrometer equipped with a cryogenic triple resonance probe. The WaterLOGSY experiments employed a 1 sec mixing time.



**Supplementary Figure S4: NMR data for the WaterLOGSY competition assay.** Experimental conditions were 10 μM DHQD, 100 μM DHS and 100 μM compounds **1**, **2** or **3** in PBS/pH 7.2, 10% 2H2O at 25˚C. Data were acquired on a Bruker 900 MHz spectrometer equipped with a cryogenic triple resonance probe with a WaterLOGSY mixing time of 2 sec.



**D**



**Supplementary Figure S5. Structure-activity relationship analysis for compounds 1-3.** (A), (B), and (C): Inhibition of the *cd*DHQD catalyzed dehydration reaction was measured for compounds **1-3** and select analogs. The critical role for binding through the phenyl aryl chlorine atoms (compounds **1** & **2**) and the carboxamideylic moiety (compound **3**) is apparent. (D) A series of analogs demonstrate that distinct additions to the dichlorobenzenesulfonamide thiazole core shared by compounds **1** and **2** have little effect on IC50.