SUPPORTING INFORMATION

In-crystal Chemical Ligation for Lead Generation

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Supplementary Methods

General

All compounds were synthesized from commercially available starting materials. The ^{1}H NMR spectra were o recorded on a Brucker DRX600. The spectra were recorded in hexadeuterodimethyl sulfoxide (DMSO-d_6, TMS internal standard). ESI-MS was obtained on a Shimadzu LCMS-2010EV.

Syntheses of ALD2-OXA9, ALD2-OXA16, ALD2-HYD6

Aldehyde (1 mmol) and hydrazine or hydroxylamine (1 mmol) were dissolved in 0.1 M HEPES-NaOH buffer, pH = 7.8 (10 ml), supplemented with 1 mM aniline. The reaction mixture was stirred for 60 min at room temperature and the mixture was purified by reversed-phase HPLC (20% – 80% CH3CN / H2O, containing 0.1 % formic acid) with UV detection at 254 nm to afford the desired compound.

(E)-2-(4-carbamimidoylbenzylideneaminooxy)acetic acid (ALD2-OXA9) : a white powder; 1H-NMR (DMSO-d_6, 600 MHz): δ 9.47 (br s, 2H), 9.26 (br s, 2H), 8.49 (s, 1H), 7.91 (d, 2H, J = 8.3 Hz), 7.84 (d, 2H, J = 8.3 Hz), 4.73 (s, 2H); ESI-MS (positive) m/z 222 $(M+H)^+$, (negative) m/z 220 $(M-H)^-$.

(E)-2-(4-carbamimidoylbenzylideneaminooxy)-2-methylpropanoic acid (ALD2-OXA16) : a white powder; 1H-NMR (DMSO-d_6, 600 MHz): δ 9.43 (br s, 2H), 9.18 (br s, 2H), 8.39 (s, 1H), 7.89 (d, 2H, J = 8.3 Hz), 7.81 (d, 2H, J = 8.3 Hz), 4.73 (s, 2H); ESI-MS (positive) m/z 250 $(M+H)^+$, (negative) m/z 248 $(M-H)^-$.

(E)-4-((2-nicotinoylhydrazono)methyl)benzimidamide (ALD2-HYD6) : a white powder; 1H-NMR (DMSO-d_6, 600 MHz): δ 10.15 (s, 1H), 9.45 (br s, 2H), 9.21 (br s, 2H), 9.13 (s, 1H), 8.80 (d, 1H, J = 4.4 Hz), 8.64 (s, 1H), 8.34 (d, 1H, J = 8.3 Hz), 7.99 (d, 2H, J = 8.3 Hz), 7.60 (dd, 1H, J = 8.3, 4.4 Hz); ESI-MS (positive) m/z 268 $(M+H)^+$, (positive) m/z 266 $(M-H)^-$.

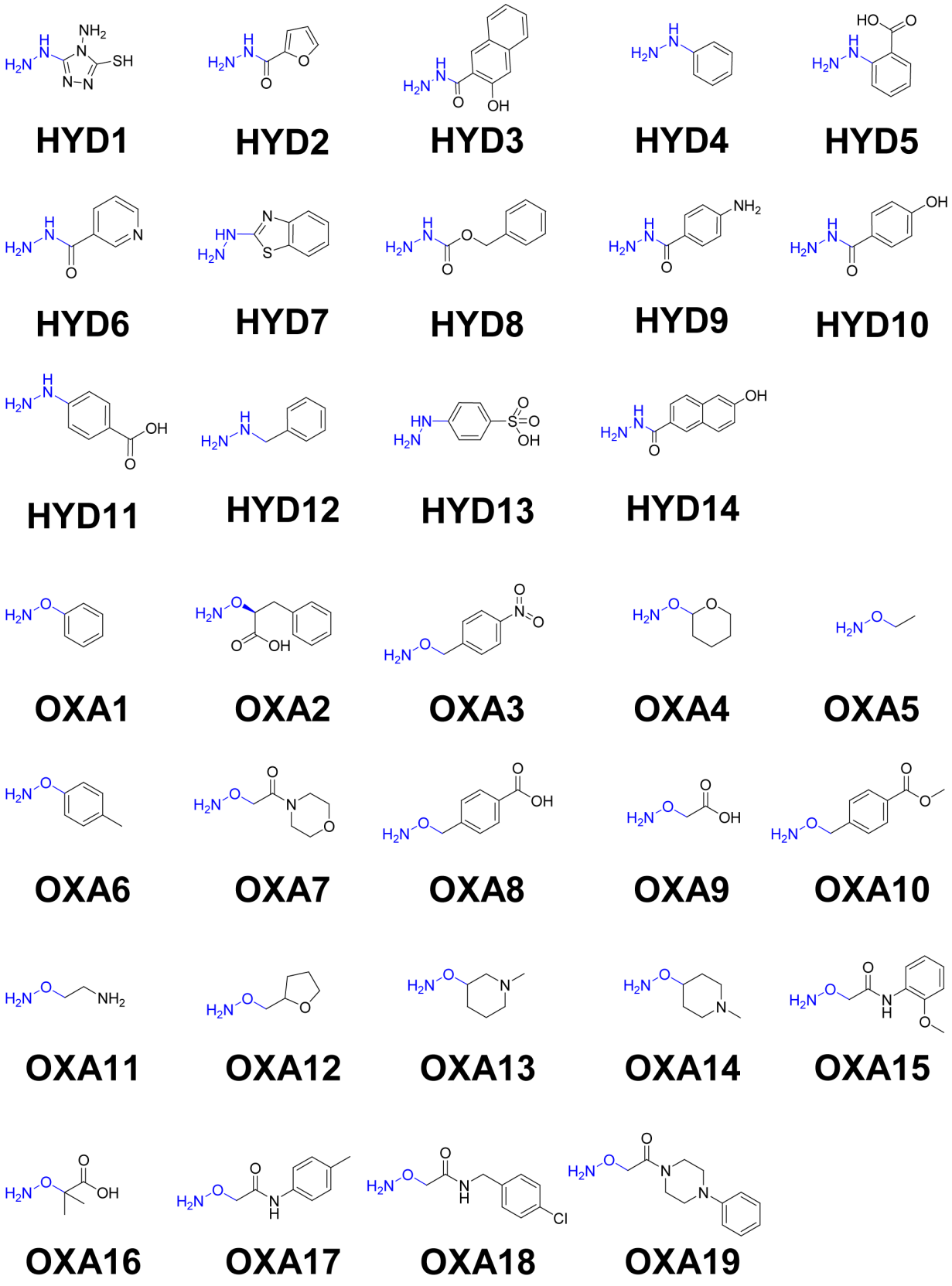
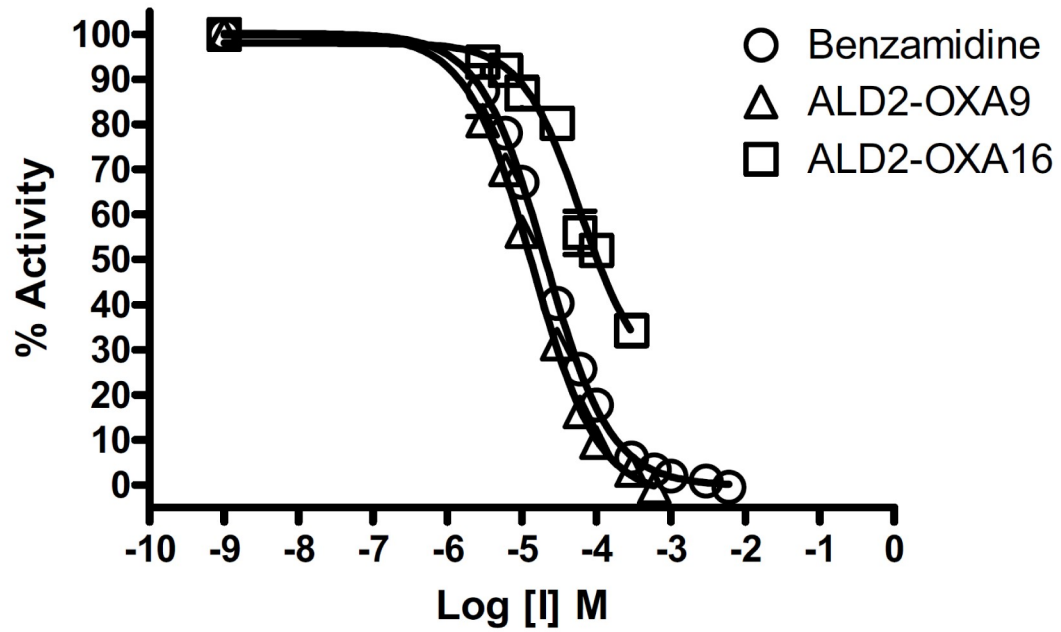
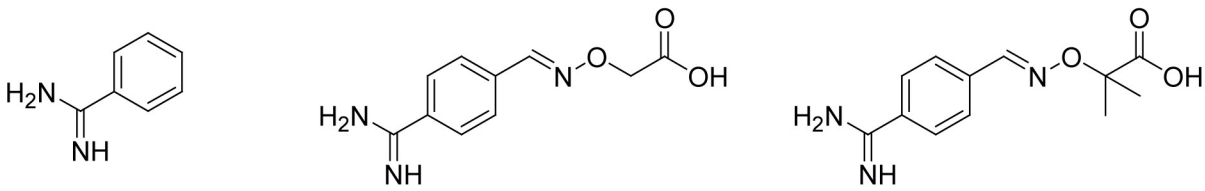


Figure S1 The focused library of tuning molecules. All compounds were obtained from commercial sources.





Hill slope = -1.0
IC_{50} = 20 μM

Hill slope = -0.99
IC_{50} = 14 μM

Hill slope = -1.1
IC_{50} = 62 μM

Figure S2 Dose-response curves of benzamidine, **ALD2-OXA9**, and **ALD2-OXA16.** The experiments and calculations of kinetic parameters were performed as described in the Methods section. To obtain the half maximal inhibitory concentration (IC_{50}), we used the percentage of inhibition (% inhibition) and the inhibitor concentration [I] as parameters for nonlinear curve fitting. The calculations were performed using the monophasic Hill equation. Obtained IC_{50} and Hill slope values were also shown. The experiment was repeated three times with essentially identical results.

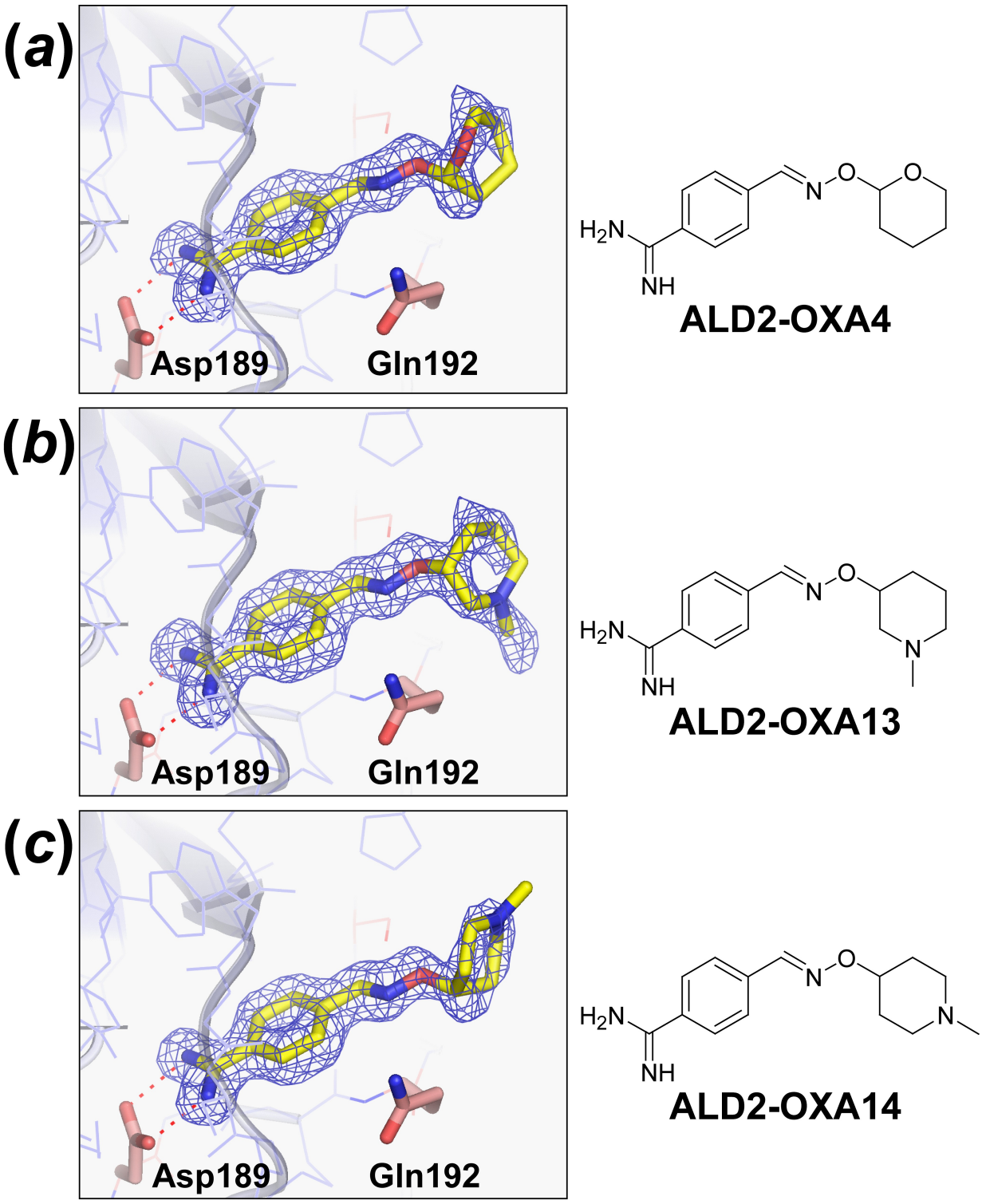


Figure S3 Marginal complex formation of self-assembled oximes. Self-assembled oximes (yellow carbon sticks) are superposed on the sigmaA-weighted Fo-Fc maps (a blue mesh contoured at 2.5 σ). Interacting side chains are shown as sticks. (*a*) **ALD2-OXA4**. (*b*) **ALD2-OXA13**. (*c*) **ALD2-OXA14**.

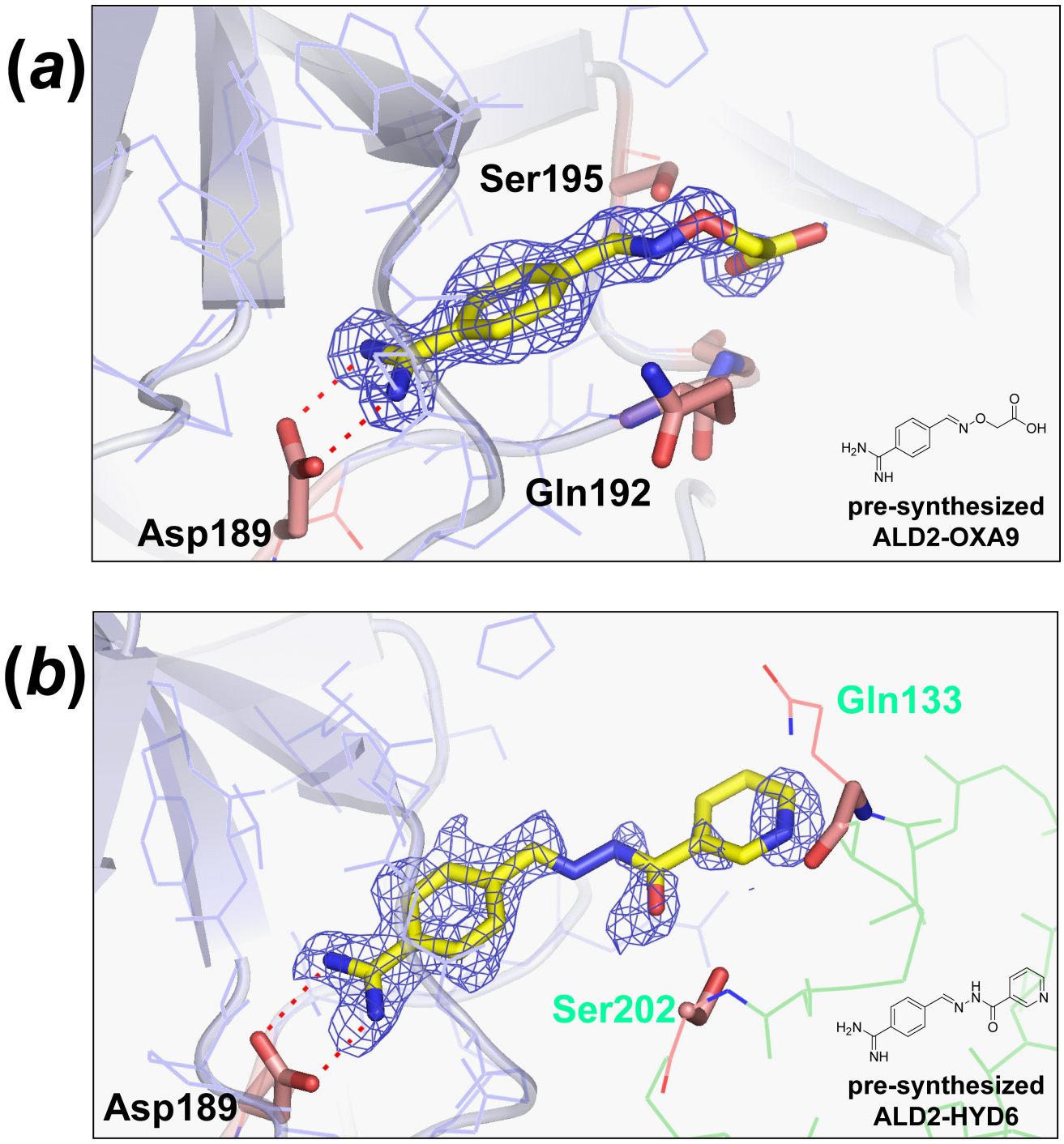


Figure S4 Binding of pre-synthesized oxime and hydrazone to the active site. **ALD2-OXA9** and **ALD2-HYD6** (yellow carbon sticks) are superposed on sigmaA-weighted Fo-Fc maps (a blue mesh contoured at 2.5 σ). Interacting side chains are shown as sticks. (*a*) Pre-synthesized **ALD2-OXA9**. (*b*) Pre-synthesized **ALD2-HYD6**. The symmetry-related counterpart is shown as green.

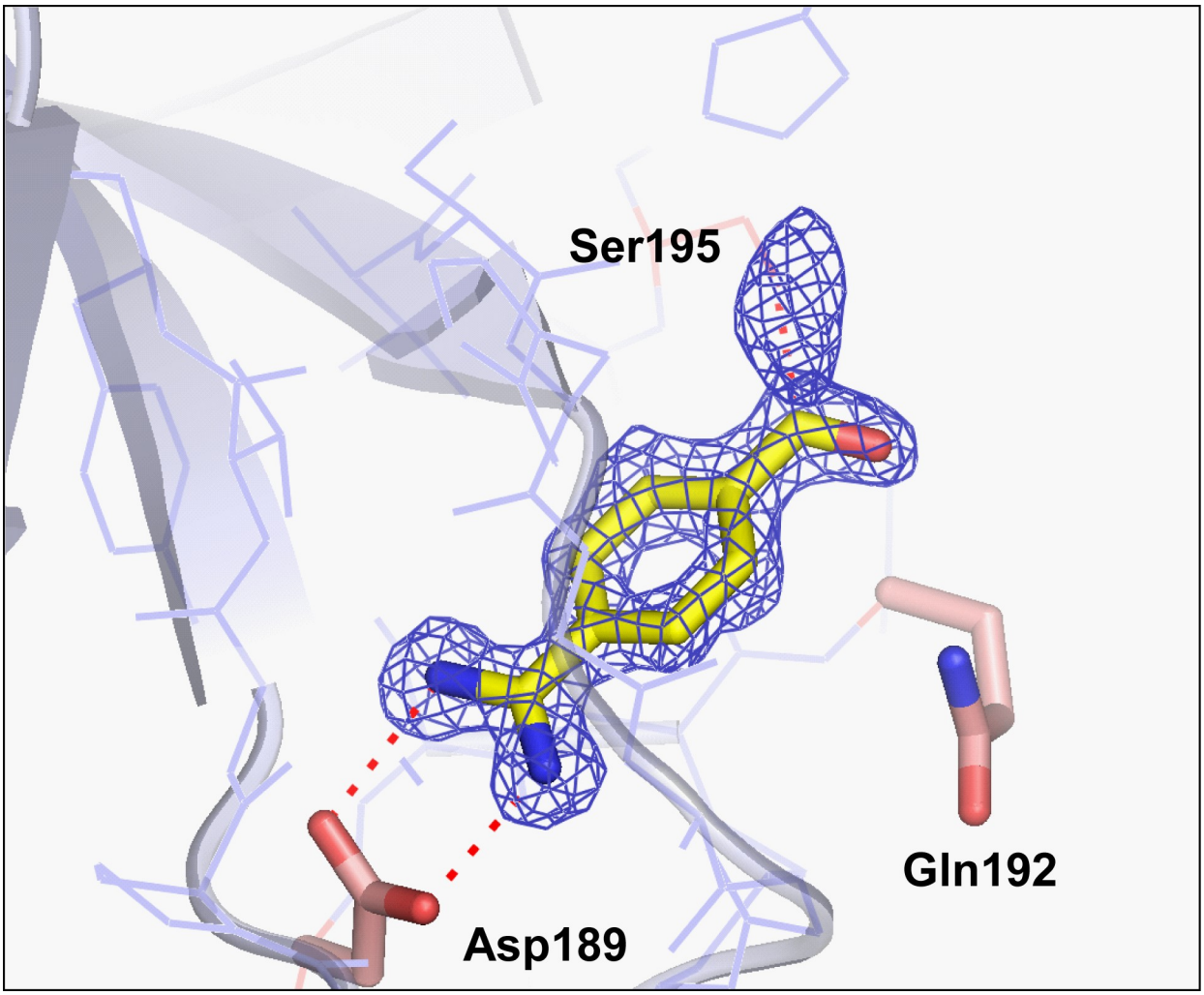


Figure S5 Blank ligation experiment without tuning molecule (but with aniline) using **ALD2**-bound crystal. Interacting side chains are shown as sticks. The sigmaA-weighted Fo-Fc map (a blue mesh contoured at 2.5σ) shows the "unknown" density blob at the expected position.

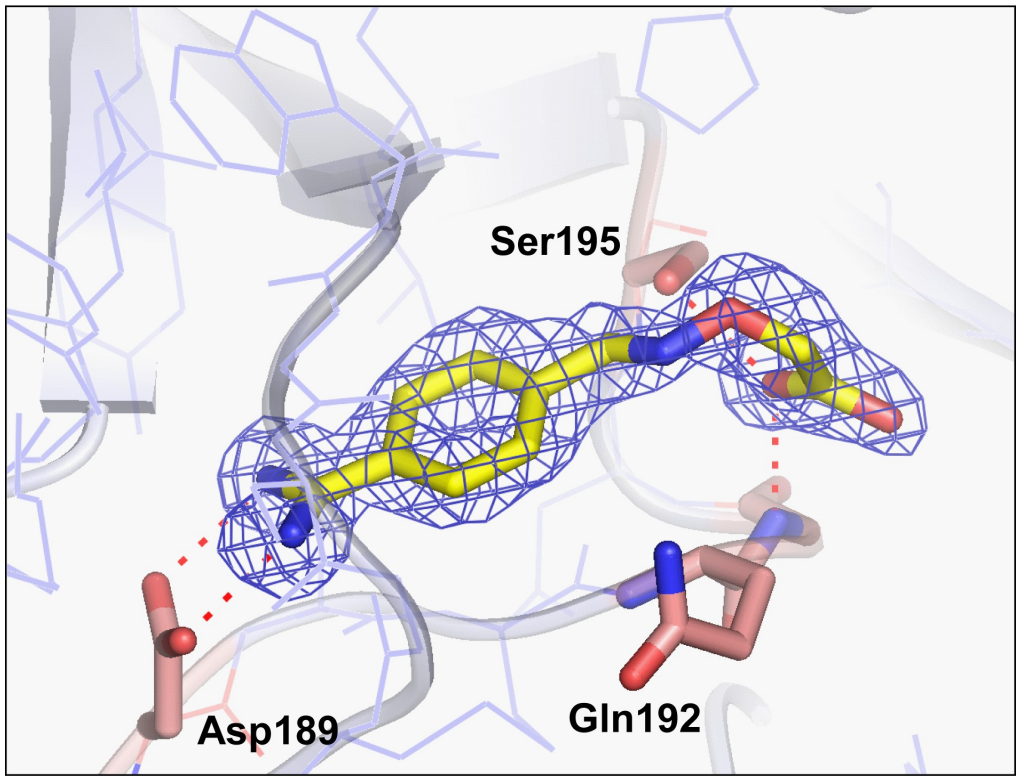


Figure S6 Selection of self-assembled oxime from cocktail solution of two tuning molecules. Top: The sigma-weighted Fo-Fc maps superposed with **ALD2-OXA9** and **ALD2-OXA16**. Bottom: The sigma-weighted Fo-Fc map (a blue mesh contoured at 2.5 σ) of cocktail experiment showing more potent inhibitor **ALD2-OXA9** (yellow carbon sticks) is bound. Interacting side chains are shown as sticks. The hydrogen bond is given by a red line.