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 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% CH$_3$CN / H$_2$O, containing 0.1 % formic acid) with UV detection at 254 nm to afford the desired compound.

(E)-2-(4-carbamimidoylbenzylideneaminoxy)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-carbamimidoylbenzylideneaminoxy)-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)$^-$. 

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