SUPPORTING INFORMATION

Structural basis of ALDH1A2 inhibition by irreversible and reversible small molecule inhibitors

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Compound properties. Compounds CM121 and 6-118 were > 95% pure by HPLC chromatography (Normal Phase; Supelcosil LC-SI, 3 uM, 4.6 x 150 mm, 2.0 ml/min 9:1 hexane:ethanol) and Reverse Phase; Beckman Coulter Ultrasphere, 5 um, 4.6 x 250 mm. 2.0 ml/min 0-7 min 60/40 to 80/20 0.1% TFA in Acetonitrile/0.1% TFA in H_2O) and gave satisfactory high resolution mass spectrometry. Analytical TLC was performed using silica gel 60 F_{254} precoated on aluminum sheets (0.20 mm thickness). ^{1}H-NMR Chemical shift values are given in ppm and splitting constants measured in Hertz. Melting point is uncorrected.

N-(4-cyanophenyl)-3-[4-(methylsulfonyl)phenyl]-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxamide (**CM121**): R_f (1:1 hexane:EtOAc) 0.71; mp 218-220 C; ^{1}H-NMR ($CDCl_3$ 500 MHz) 8.52 (s, 1H), 7.99 (dd, 4H, J=8.5, 15), 7.88 (d, 2H, J=8.5), 7.77 (d, 2H, J=8.5), 7.43 (d, 1H, J=11), 7.37 (bs, 1H), 7.20 (m, 1H), 6.89 (d, 1H, J=8), 6.80 (m, 1H), 3.05 (s, 3H); ESMS 459 (M-H, 100%); HRMS $C_{24}H_{18}FN_4O_3S$ calc 459.0927, found 459.0922.

{4-[4-nitro-3-(pyrrolidin-1-yl)phenyl]piperazin-1-yl}(3-ethoxythiophen-2-yl)methanone (**6-118**): R_f (1:1 hexane:EtOAc) 0.45; ^{1}H-NMR ($CDCl_3$ 300 MHz) 7.78 (d, 1H, J=9.3), 7.30 (d, 1H, J=5.4), 6.70 (d, 1H, J=5.4), 6.22 (dd, 1H, J=2.7, 9.3), 6.08 (d, 1H, J=2.1), 4.07 (q, 2H, J=6.9), 3.71 (m, 4H), 3.33 (m, 4H), 3.17 (m, 4H), 1.90 (m, 4H), 1.34 (t, 3H, J=6.9); HRMS $C_{21}H_{27}N_4O_4S$ calc 431.1753, found 431.1756.

Table S1: X-ray data collection and refinement statistics

Structure	ALDH1A2-NAD-WIN18, 446	ADLH1A2-NAD-6118	ALDH-NAD-CM121	ALDH-CM121
Data Collection				
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions	a=81.96, b=139.82, c=84.90 Å α=γ=90.00, β=94.07	a=84.60, b=140.52, c=164.61 Å α= β= γ=90.00	a=84.85, b= 141.86, c=164.75 α= β= γ=90.00	a=84.83, b= 139.47, c=163.07 α= β= γ=90.00
Resolution range	40.8-1.89 (1.89-2.0)	51.1-2.20 (2.2-2.4)	72.8-2.30 (2.3-2.4)	81.5-2.60 (2.6-2.7)
Unique reflections	150,515 (23,625)	98,817 (22,278)	88,789 (10,489)	59,848 (6,298)
Completeness (%)	99.0 (99.7)	98.7 (97.9)	99.8 (99.6)	99.3 (99.2)
I/σI	16.3 (5.04)	13.9 (4.50)	17.8 (3.76)	14.6 (5.07)
R_{sym}[a](%)	6.6 (31.2)	10.8 (56.3)	7.9 (64.2)	12.3 (66.9)
Structure refinement				
Non-solvent atoms	15548	15540	15552	15376
Solvent molecules	2169	712	382	194
Average B-factor all (Å^2)	17.4	30.8	42.3	45.3
Average B-factor protein (Å^2)	16.1	30.7	42.2	45.3
Average B-factor NAD (Å^2)	22.1	29.2	49.5	---
Average B-factor inhibitor (Å^2)	27.6	41.9	59.3	57.2
Average B-factor solvent (Å^2)	27.6	32.4	38.9	36.8
Rcryst[b] (%)	13.8	20.3	20.6	22.2
Rfree[c] (%)	17.8	26.9	26.4	27.7
Rfree reflection set size	1504 (1.0 %)	988 (1.0 %)	1775 (2.0 %)	1076 (1.8 %)
r.m.s.d.[d] bonds (Å)	0.008	0.003	0.002	0.004
r.m.s.d angles (°)	0.889	0.834	0.541	0.610
Wilson B (Å^2)	16.1	27.8	37.9	36.2
Coordinate error (Å)	0.16	0.30	0.32	0.34
Ramachadran favored (%)	97.8	95.0	94.0	92.9
Ramachadran allowed (%)	2.2	4.4	4.5	5.9

[a] Rsym = 100 xΣhΣi|Ihi-Ih|/ΣhiI where h are unique reflection indices.

[b] Rcryst = 100 xΣ|Fobs-Fmodel|/F obs where Fobs and Fmodel are observed and calculated structure actor amplitudes, respectively.

[c] Rfree is Rcryst calculated for randomly chosen unique reflections, which were excluded from the refinement.

[d] r.m.s.d. = root mean square deviation from ideal values.

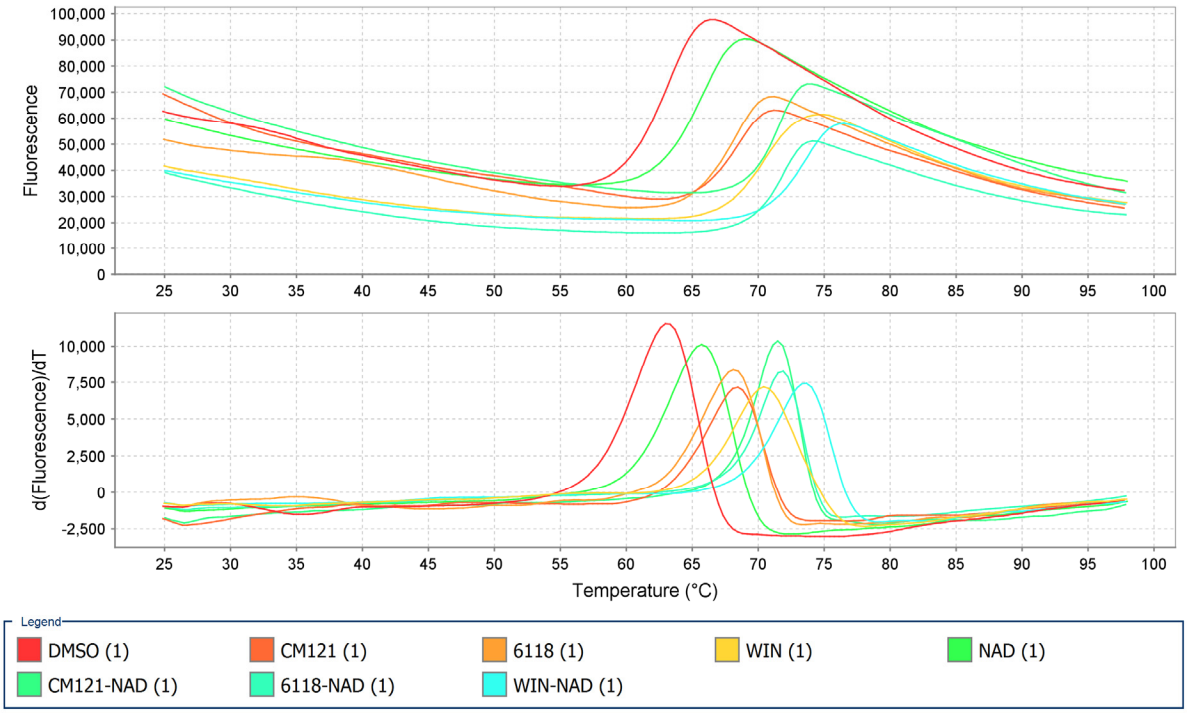

Fig. S1: **Representative DSF data of ALDH1A2-ligand complexes.** Top: Melt curve plot. Bottom: Derivative plot.

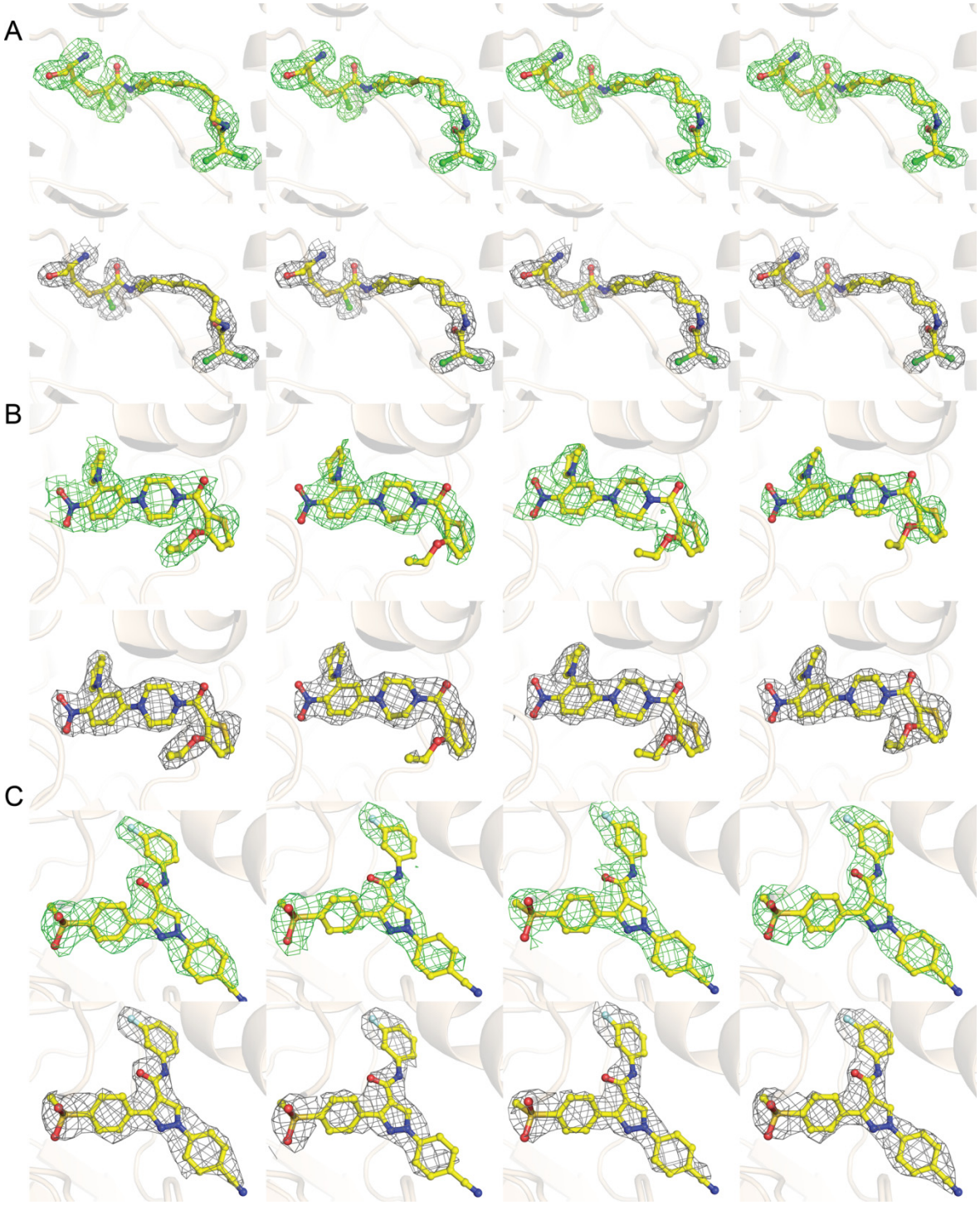

Fig. S2: Electron density maps of inhibitors in all four active sites of the ALDH1A2 tetramer. (A) WIN18,446, (B) 6-118 and (C) CM121. The upper panels show the Fo-Fc density maps (contoured at 2σ) omitting the inhibitor during the last refinement cycle, the bottom panels show the 2Fo-Fc density maps (contoured at 1σ) of the completed refinement.

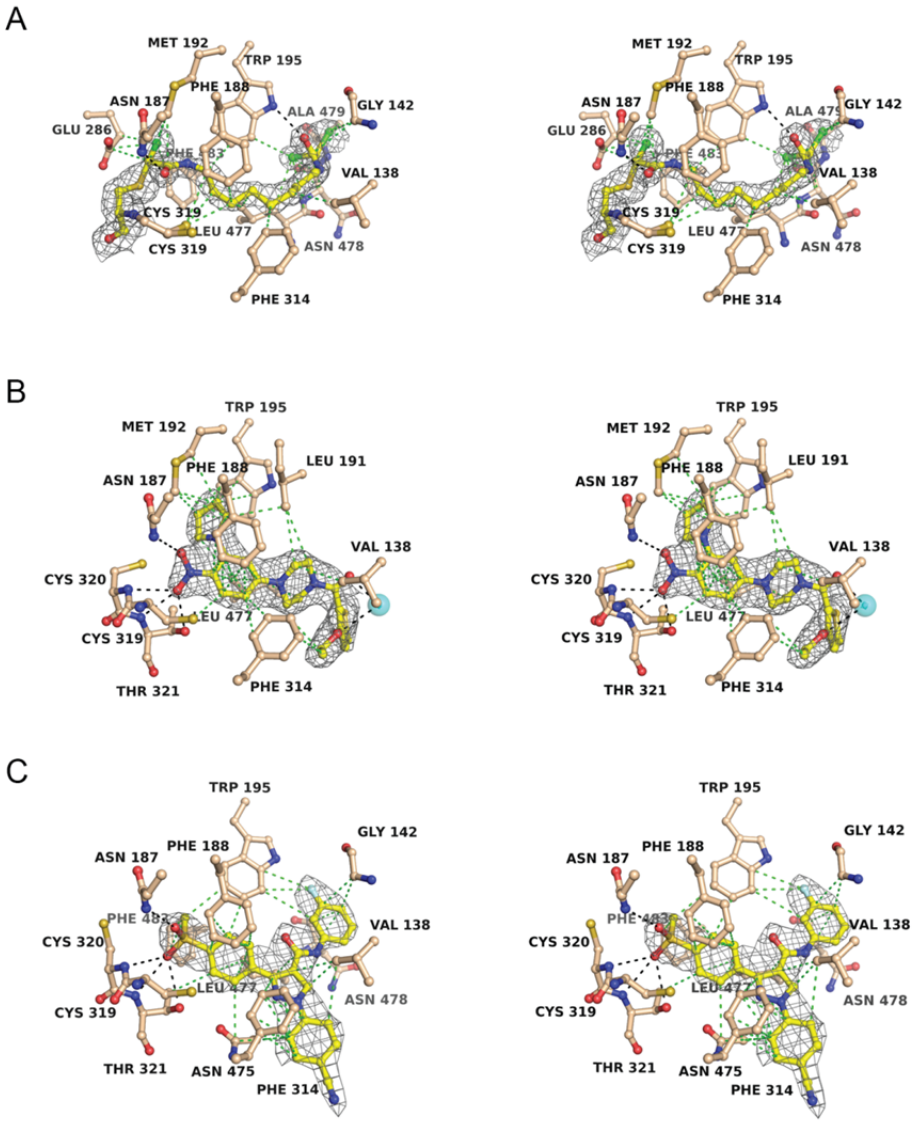

Fig. S3: Stereo presentations of the binding interactions of inhibitors in ALDH1A2. (A) WIN18,446, (B) 6-118 and (C) CM121.