Progresses in the Field of Aldehyde Dehydrogenase Inhibitors: Novel Imidazo[1,2-*a*]pyridines Against the 1A Family.

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Table 1-SI. Data collection and refinement statistics of the human ALDH1A3 model.

DATA COLLECTION	**3c**
Space group	$P2_122_1$
Cell dimensions	
a, b, c (Å)	81.3 89.2 159.2
α, β, γ (°)	90 90 90
Resolution (Å)	47.96 - 2.9
R_{pim} / R_{merge}	0.061 (0.40)/0.117(0.80)
Mean(I) / sd(I)	2.3 (2.1)
Completeness (%)	98.5 (98.95)
Redundancy	10.6 (4.6)
REFINEMENT	
Resolution (Å)	2.9
No. reflections	25959 (2556)
R_{work} / R_{free}	0.19 / 0.26(0.30 / 0.35)
No. atoms	
Protein	7408
Solvent	11
Ligands	138
Mean B-factors	
Protein (Å^2)	60.9
Water (Å^2)	44.5
Ligands (Å^2)	79.4
R.m.s deviations	
Bond lengths (Å)	0.017
Angles (°)	1.57
Ramachandran outliers (%)	0.1

Figure 1-SI. All-atom root mean square deviation (RMSD) of compound **3f** (red) and **3f-**ALDH1A3 complex (black) along MD trajectory.

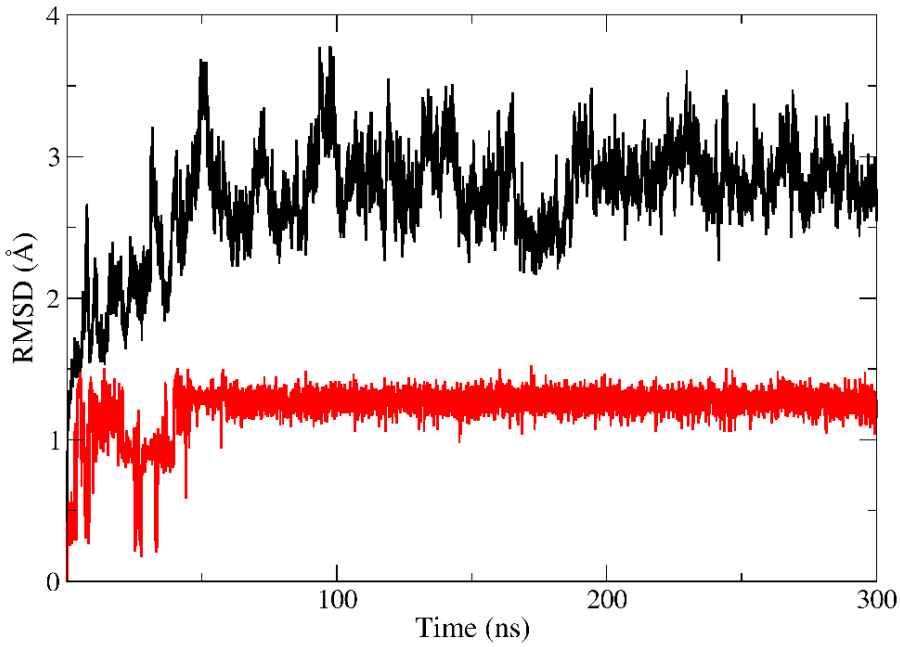

Figure 2-SI. Overlap of the binding mode of the crystallized compound **3c** (aquamarine sticks) and the representative poses of compound **3f** (green sticks), extrapolated from MD trajectories.

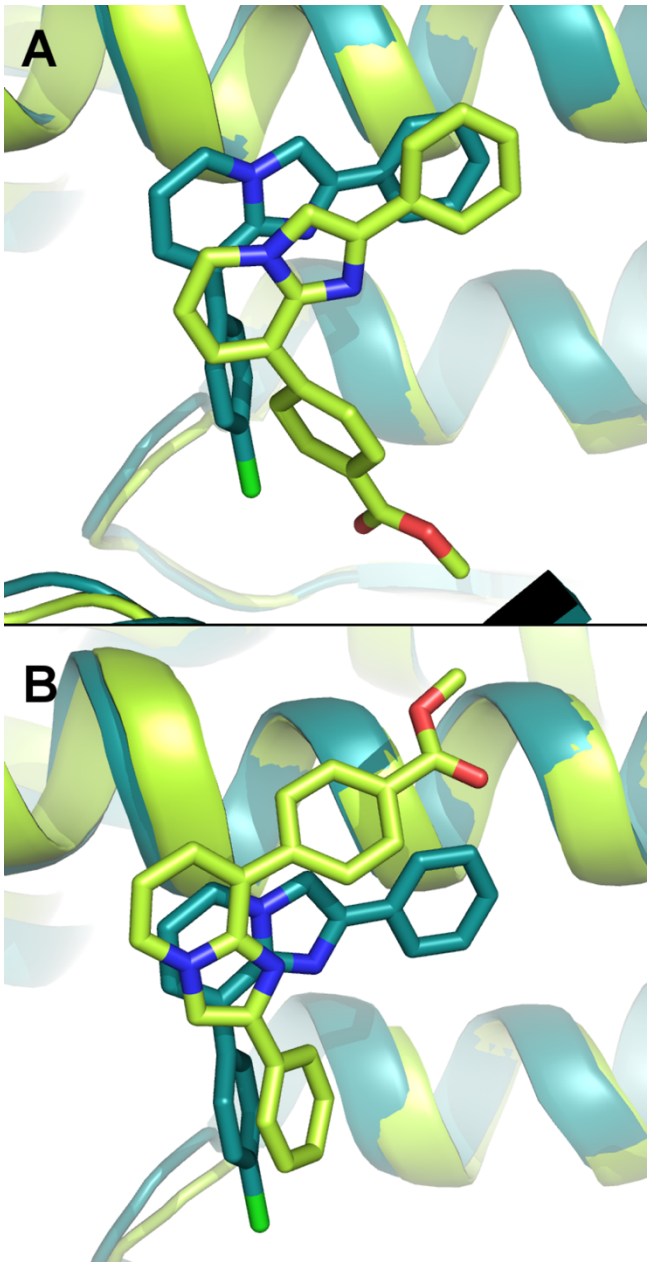

Figure 3-SI. Anti-proliferative activity of compound **3c** against U87-MG cell line.

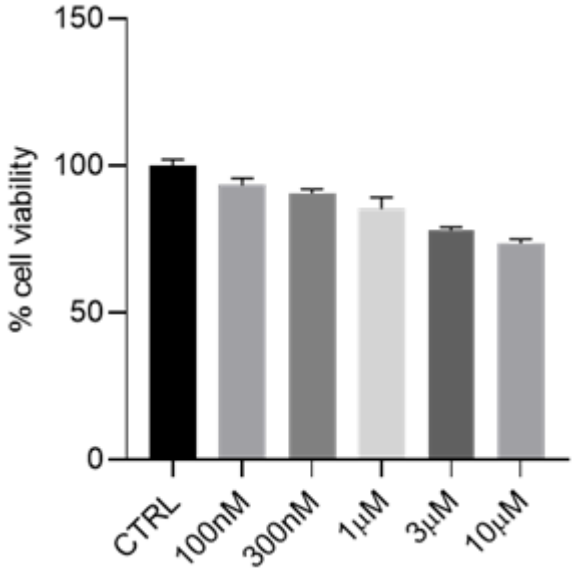

Table 2-SI. Physio-chemical and spectral data of compounds **2b**,**c**.

8-Bromo-2-(4-methylphenyl)imidazo[1,2-a]pyridine, 2b. White solid. M.p. 258-260 °C. Cryst. solvent: Acetonitrile. Yield: 55%. ^{1}H-NMR (δ, ppm): 8.953 (d, 1H, J=1.20 Hz), 8.603 (s, 1H), 8.321 (d, 2H, J = 8.88 Hz), 8.242 (d, 2H, J = 8.88 Hz), 7.638 (d, 1H, J = 9.60 Hz), 7.445 (dd, 1H, J = 9.60 Hz, J= 1.71 Hz).
8-Bromo-2-(4-methoxyphenyl)imidazo[1,2-a]pyridine, 2c. White solid. M.p. 232-234 °C. Cryst. solvent: EtOH. Yield: 76%. ^{1}H-NMR (δ, ppm): 9.189 (s, 1H), 8.678 (s, 1H), 7.928 (d, 1H, J = 9.28 Hz), 7.853 (d, 1H, J = 9.44 Hz), 7.557 (s, 1H), 7.550 (d, 1H, J = 7.95 Hz), 7.499 (t, J = 8.09 Hz, 1H), 7.091 (d, 1H, J = 7.92 Hz), 3.867 (s, 3H).

Table 3-SI. Physio-chemical and spectroscopic data of compounds **3a-t**.

2,8-Diphenylimidazo[1,2-*a*]pyridine, **3a**. M.p. 86-89°C. Cryst. solvent: Methanol. Yield: 16%. ^{1}H-NMR (δ, ppm): 8.558 (d, 1H, J = 6.70 Hz), 8.507 (s, 1H), 8.224 (d, 2H, J = 7.82 Hz), 8.000 (d, 2H, J = 7.70 Hz), 7.547 (t, 2H, J = 7.11 Hz), 7.471 (m, 5H), 7.337 (t, 1H, J = 7.37 Hz), 7.022 (t, 1H, J = 6.91 Hz). ^{13}C-NMR (δ, ppm): 144.75, 143.84, 136.61, 134.31, 129.19, 129.12, 128.78, 128.59, 128.21, 126.60, 126.11, 123.51, 113.03, 110.19. HRMS (ESI, m/z): calcd for $C_{19}H_{14}N_2$ 270.11515, found $[M+H]^+$ 271.12297.
8-(4-Fluorophenyl)-2-phenylimidazo[1,2-*a*]pyridine, **3b**. M.p. 129-132°C. Cryst. solvent: Methanol. Yield: 32%. ^{1}H-NMR (δ, ppm): 8.559 (d, 1H, J = 6.70 Hz), 8.509 (s, 1H), 8.319 (dd, 2H, J = 6.17 Hz, J = 4.52 Hz), 8.003 (d, 2H, J = 7.74 Hz), 7.485 (m, 3H), 7.365 (m, 3H), 7,021 (t, 1H, J = 6.94 Hz). ^{13}C-NMR (δ, ppm): 163.72, 144.77, 134.27, 132.94, 131.18, 129.17, 128.23, 127.38, 126.65, 126.13, 123.38, 115.72, 115.51, 112.99, 110.25. HRMS (ESI, m/z): calcd for $C_{19}H_{13}FN_2$ 288.10573, found $[M+H]^+$ 289.11355.
8-(4-Chlorophenyl)-2-phenylimidazo[1,2-*a*]pyridine, **3c**. M.p. 150-153°C. Cryst. solvent: Methanol. Yield: 42%. ^{1}H-NMR (δ, ppm): 8.578 (d, 1H, J = 6.73 Hz), 8.518 (s, 1H), 8.304 (d, 2H, J = 8.64 Hz), 8.004 (d, 2H, J = 7.12 Hz), 7.615 (d, 2H, J = 8.64 Hz), 7.552 (d, 1H, J = 6.70 Hz), 7.167 (t, 2H, J = 7.64 Hz), 7.343 (t, 1H, J = 7.37 Hz), 7.030 (t, 1H, J = 6.95 Hz). ^{13}C-NMR (δ, ppm): 144.80, 143.58, 135.34, 134.22, 133.27, 130.81, 129.18,128.29, 128.27, 127.08,126.97, 126.14, 123.61, 112.98, 110.29. HRMS (ESI, m/z): calcd for $C_{19}H_{13}ClN_2$ 304.07618, found $[M+H]^+$ 305.08400.
8-(4-Isobutylphenyl)-2-phenylimidazo[1,2-*a*]pyridine, **3d**. M.p. 83-85°C. Cryst. solvent: Ethanol. Yield: 43%. ^{1}H-NMR (δ, ppm): 8.531 (d, 1H, J = 6.68 Hz), 8.494 (s, 1H), 8.159 (d, 2H, J = 8.2 Hz), 7.998 (d, 2H, J = 7.00 Hz), 7.492-7.446 (m, 3H), 7.375-7.322 (m, 3H), 7.005 (t, 1H, J = 6.96 Hz), 3.309 (s, 3H), 2.569 (d, 2H, J = 7.24 Hz), 1.967-1.900 (m, 1H), 0.936 (d, 6H, J = 6.6 Hz).

^{13}C-NMR (δ, ppm): 144.66, 143.87, 141.70, 134.34, 134.03, 129.36, 129.18, 128.85, 128.54, 128.18, 126.30, 126.11, 123.08, 113.05, 110.14, 44.87, 30.09, 22.73. HRMS (ESI, m/z): calcd for $C_{23}H_{22}N_2$ 326.17775, found $[M+H]^+$ 327.18558.
1-Phenyl-2-(4-(2-phenylimidazo[1,2-*a*]pyridin-8-yl)phenoxy)ethan-1-one, **3e**. M.p. 82-85°C. Cryst. solvent: Ethanol. Yield: 10%. ^{1}H-NMR (δ, ppm): 8.480 (dd, 1H, J = 7.35 Hz, J = 1.23 Hz), 8.260 (s, 1H), 8.140 (d, 2H, J = 7.27 Hz), 8.030 (d, 2H, J = 7.25 Hz), 7.680 (t, 1H, J = 7.00 Hz), 7.570 (d, 2H, J = 7.15 Hz), 7.500 (t, 3H, J = 7.20 Hz), 7.300 (d, 2H, J = 7.10 Hz), 7.210 (dd, 1H, J = 7.15 Hz, J = 1.20 Hz), 6.980 (d, 2H, J = 7.00 Hz), 6.860 (t, 1H, J = 7.20 Hz), 5.70 (s, 2H). ^{13}C-NMR (δ, ppm): 194.53, 147.21, 143.88, 139.59, 134.83, 134.30, 129.34, 129.11, 128.39, 128.04, 125.95, 120.34, 112.62, 110.40, 104.34, 71.38. HRMS (ESI, m/z): calcd for $C_{21}H_{16}N_2O_2$ 328.12063, found $[M+H]^+$ 329.12845.
Methyl 4-(2-phenylimidazo[1,2-*a*]pyridin-8-yl)benzoate, **3f**. M.p. 190 °C. Cryst. solvent: Ethanol. Yield: 39%. ^{1}H-NMR (δ, ppm): 8.619 (d, 1H, J = 6.16 Hz), 8.539 (s, 1H), 8.423 (d, 2H, J = 8.57 Hz), 8.128 (d, 2H, J = 8.52 Hz), 8.017 (d, 2H, J = 7.74 Hz), 7.634 (dd, 1H, J = 0.96 Hz, J = 7.04 Hz), 7.473 (t, 2H, J = 7.40 Hz), 7.349 (t, 1H, J = 7.40 Hz), 7.064 (t, 1H, J = 6.92 Hz), 2.664 (s, 3H). ^{13}C-NMR (δ, ppm): 198.10, 144.90, 143.56, 141.06, 136.51, 134.16, 129.23, 129.20, 128.68, 128.32, 127.44, 127.23, 126.16, 124.35, 112.98, 110.32, 49.07. HRMS (ESI, m/z): calcd for $C_{21}H_{16}N_2O_2$ 328.12120, found $[M+H]^+$ 329.12817.
8-([1,1'-Biphenyl]-4-yl)-2-phenylimidazo[1,2-*a*]pyridine, **3g**. M.p. 204-205°C. Cryst. solvent: Ethanol. Yield: 34%. ^{1}H-NMR (δ, ppm): 8.577 (d, 1H, J = 6.25 Hz), 8.526 (s, 1H), 8.373 (d, 2H, J = 8.40 Hz), 8.026 (d, 2H, J = 7.12 Hz), 7.866 (d, 2H, J = 8.48 Hz), 7.800 (d, 2H, J = 7.21 Hz), 7.588 (d, 1H, J = 7.92 Hz), 7.544-7,458 (m, 4H), 7.414 (t, 1H, J = 7.33 Hz), 7.348 (t, 1H, J = 4.40 Hz), 7.048 (t, 1H, J = 6,96 Hz). ^{13}C-NMR (400 MHz, DMSO-d6) (δ, ppm): 144.22, 143.29, 139.61, 135.11, 133.78, 129.09, 128.96, 128.66, 127.70, 127.55, 126.58, 126.47, 126.13, 125.61, 122.84, 112.54, 109.70. HRMS (ESI, m/z): calcd for $C_{25}H_{18}N_2$ 346.14645, found $[M+H]^+$ 347.15428.

8-Phenyl-2-(p-tolyl)imidazo[1,2-*a*]pyridine, **3h**. M.p. 40°C. Cryst. solvent: Methanol. Yield: 40%. ^{1}H-NMR (δ, ppm): 8.537 (d, 1H, J = 5.20 Hz), 8.450 (s, 1H), 8.111 (d, 2H, J = 7.78 Hz), 7.882 (d, 2H, J = 8.09 Hz), 7.543 (t, 2H, J = 7.54 Hz), 7.478 (dd, 1H, J = 7.12 Hz, J = 1.04 Hz), 7,444 (t, 1H, J = 7.36 Hz), 7.267 (d, 2H, J = 7.93 Hz), 7.004 (t, 1H, J = 6.90 Hz), 2.344 (s, 3H). ^{13}C-NMR (δ, ppm): 144.38, 143.25, 136.98, 136.17, 131.05, 129.25, 128.62, 128.27, 128.04, 127.99, 126.02, 125.55, 122.86, 112.41, 109.24, 20.86. HRMS (ESI, m/z): calcd for $C_{20}H_{16}N_2$ 284.13080, found $[M+H]^+$ 285.13862.

8-(4-Fluorophenyl)-2-(p-tolyl)imidazo[1,2-*a*]pyridine, **3i**. M.p. 125-128°C. Cryst. solvent: Ethanol. Yield: 31%. ^{1}H-NMR (δ, ppm): 8.544 (d, 1H, J = 6.66 Hz), 8.460 (s, 1H), 8.301 (dd, 2H, J = 8.84 Hz, J = 5.64 Hz), 7.888 (d, 2H, J = 8.09 Hz), 7.499 (d, 1H, J = 6.33 Hz), 7.385 (t, 2H, J = 8.96 Hz), 7.273 (d, 2H, J = 7.88 Hz), 7.012 (t, 1H, J = 6.92 Hz), 2.348 (s, 3H). ^{13}C-NMR (δ, ppm): 144.88, 143.62, 137.53, 132.98, 131.48, 131.18, 129.76, 127.26, 126.57, 126.05, 123.28, 115.73, 115.51, 112.90, 109.81, 21.36. HRMS (ESI, m/z): calcd for $C_{20}H_{15}FN_2$ 302.12138, found $[M+H]^+$ 303.12920.

8-(4-Chlorophenyl)-2-(p-tolyl)imidazo[1,2-*a*]pyridine, **3j**. M.p. 178-180°C. Cryst. solvent: Methanol. Yield: 40%. ^{1}H-NMR (δ, ppm): 8.559 (d, 1H, J = 6.68 Hz), 8.463 (s, 1H), 8.294 (d, 2H, J = 6.72 Hz), 7.890 (d, 2H, J = 8.09 Hz), 7.613 (d, 2H, J = 6.70 Hz), 7.533 (dd, 1H, J = 7.16 Hz, J = 1.04 Hz,), 7.273 (d, 2H, J = 7.89 Hz), 7.015 (t, 1H, J = 6.92 Hz), 2.349 (s, 3H). ^{13}C-NMR (δ, ppm): 145.48, 145.10, 138.60, 137.47, 136.67, 134.75, 129.62, 129.37, 128.02, 126.08, 125.70, 113.44, 112.68, 107.60, 21.36. HRMS (ESI, m/z): calcd for $C_{20}H_{15}ClN_2$ 318.09183, found $[M+H]^+$ 319.09965.

8-(4-Fluorophenyl)-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine, **3k**. M.p. 167°C. Cryst. solvent: Ethanol. Yield: 41%. ^{1}H-NMR (δ, ppm): 8.549 (dd, 1H, J = 1.08 Hz, J = 7.16 Hz), 8.404 (s, 1H), 8.296 (dd, 2H, J = 2.00 Hz, J = 6.64 Hz), 7.924 (dd, 2H, J = 2.04 Hz, J = 6.72 Hz), 7.606 (dd, 2H, J = 2.00 Hz, J =6.64 Hz), 7.521 (dd, 1H, J = 1.08 Hz, J = 7.16 Hz), 7.048-6.985 (m, 3H),

3.808 (s, 3H). ^{13}C-NMR (δ, ppm): 159.58, 144.87, 143.46, 135.42, 133.20, 130.80, 128.78, 127.47, 126.81, 123.36, 115.70, 115.49, 114.62, 112.78, 109.22, 55.63. HRMS (ESI, m/z): calcd for $C_{20}H_{15}FN_2O$ 318.11629, found $[M+H]^+$ 319.12412.
8-(4-Chlorophenyl)-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine, **3l**. M.p. 167°C. Cryst. solvent: Ethanol. Yield: 20%. ^{1}H-NMR (δ, ppm): 8.549 (dd, 1H, J = 1.00 Hz, J = 7,12 Hz), 8.403 (s, 1H), 8.298 (d, 2H, J = 8.642 Hz), 7.926 (d, 2H, J = 8.803 Hz), 7.606 (d, 2H, J = 8.683 Hz), 7,523 (dd, 1H, J = 7.12 Hz, J = 1.00 Hz), 7.043-6.986 (m, 3H), 3.810 (s, 3H). ^{13}C-NMR (δ, ppm): 159.58, 144.87, 143.46, 135.42, 133.20, 130.80, 128.77, 126.82, 126.80, 123.36, 114.61, 112.77, 109.21, 55.62. HRMS (ESI, m/z): calcd for $C_{20}H_{15}ClN_2O$ 334.08674, found $[M+H]^+$ 335.09457.
8-(3,5-Dimethoxyphenyl)-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine, **3m**. M.p. 122-125 °C. Cryst. solvent: Methanol. Yield: 36%. ^{1}H-NMR (δ, ppm): 8.529 (d, 1H, J = 5.92 Hz), 8.388 (s, 1H), 7.924 (d, 2H, J = 8.76 Hz), 7.548 (d, 1H, J = 6.36 Hz), 7.040 (d, 2H, J = 8.76 Hz), 6.970 (t, 1H, J = 6.92 Hz), 6.590 (s, 1H), 3.854 (s, 3H), 3.808 (s, 6H). ^{13}C-NMR (δ, ppm): 160.22, 159.08, 144.23, 143.18, 137.91, 127.39, 126.88, 126.46, 126.12, 122.83, 114.21, 112.22, 108.60, 106.78, 100.11, 55.26, 55.14. HRMS (ESI, m/z): calcd for $C_{22}H_{20}N_2O_3$ 360.14684, found $[M+H]^+$ 361.15467.
2-(4-Methoxyphenyl)-8-(3,4,5-trimethoxyphenyl)imidazo[1,2-*a*]pyridine, **3n**. M.p. 145-146 °C. Cryst. solvent: Methanol. Yield: 36%. ^{1}H-NMR (δ, ppm): 8.515 (d, 1H, J = 5.76 Hz), 8.391 (s, 1H), 7.943 (d, 2H, J = 8.84 Hz), 7.699 (s, 2H), 7.585 (dd, 1H, J = 7.28 Hz, J = 1.00 Hz), 7.037 (d, 2H, J = 8.84 Hz), 6.994 (t, 1H, J = 6.92 Hz), 3.924 (s, 6H), 3.832 (s, 3H), 3.758 (s, 3H). ^{13}C-NMR (δ, ppm): 159.56, 153.09, 145.57, 144.22, 138.33, 132.62, 131.87, 127.28, 126.64, 124.39, 122.14, 114.08, 112.33, 107.62, 106.47, 60.94, 56.28, 55.32. HRMS (ESI, m/z): calcd for $C_{23}H_{22}N_2O_4$ 390.15741, found $[M+H]^+$ 391.16523.
Methyl 4-(2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridin-8-yl)benzoate, **3o**. M.p. 130°C. Cryst. solvent: Methanol. Yield: 38%. ^{1}H-NMR (δ, ppm): 8.591 (d, 1H, J = 6.48 Hz), 8.425-8.404 (m, 3H), 8.121 (d, 2H, J = 8.49 Hz), 7.938 (d, 2H, J = 8.80 Hz), 7.605 (d, 1H, J = 6.48 Hz), 7.054-7.019

(m, 3H), 3.812 (s, 3H), 3,337 (s, 3H). ^{13}C-NMR (δ, ppm): 198.08, 159.60, 144.97, 143.45, 141.17, 136.47, 129.23, 128.68, 127.49, 127.30, 126.97, 126,77, 124.1, 114.63, 112.78, 109.25, 55.63, 27.30. HRMS (ESI, m/z): calcd for $C_{22}H_{18}N_2O_3$ 342.13628, found $[M+H]^+$ 343.14410.

8-([1,1'-Biphenyl]-4-yl)-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine, **3p**. M.p. 208-210°C. Cryst. solvent: Methanol. Yield: 46%. ^{1}H-NMR (δ, ppm): 8.548 (d, 1H, J = 6,72 Hz), 8,411 (s, 1H), 8.364 (d, 2H, J = 8.48 Hz), 7.946 (d, 2H, J = 8.80 Hz), 7.858 (d, 2H, J = 7.20 Hz), 7.796 (d, 2H, J = 7.20 Hz), 7.5663-7.5039 (m, 3H), 7.413 (t, 1H, J = 7.33 Hz), 7.053-7.003 (m, 3H), 3.815 (s, 3H). ^{13}C-NMR (δ, ppm): 159.55, 144.84, 143.70, 140.14, 135.74, 129.61, 129.49, 128.07, 127.72, 127.46, 127.11, 126.99, 126.93, 126.5, 123.12, 114.63, 112.86, 109.16, 55.63. HRMS (ESI, m/z): calcd for $C_{26}H_{20}N_2O$ 376.15701, found $[M+H]^+$ 377.16484.

8-(4-Fluorophenyl)-2-(3-methoxyphenyl)imidazo[1,2-*a*]pyridine, **3q**. M.p. 110-113°C. Cryst. solvent: Ethanol. Yield: 53%. ^{1}H-NMR (δ, ppm): 8.546 (dd, 1H, J = 6.66 Hz, J = 1.02 Hz), 8.529 (s, 1H), 8.316 (dd, 2H, J = 7.86 Hz, J = 4.54 Hz), 7.548 (m, 3H), 7.385 (m, 3H), 7.020 (t, 1H, J = 9.07 Hz), 6.922 (dd, 1H, J = 7.82 Hz, J = 2.18 Hz), 3.836 (s, 3H). ^{13}C-NMR (δ, ppm): 159.59, 144.10, 135.16, 132.40, 130.68, 129.79, 126.86, 126.14, 122.91, 118.06, 115.24, 115.02, 113.13, 112.54, 111.18, 110.06, 55.07. HRMS (ESI, m/z): calcd for $C_{20}H_{15}FN_2O$ 318.11629, found $[M+H]^+$ 319.12412.

8-(4-Chlorophenyl)-2-(3-methoxyphenyl)imidazo[1,2-*a*]pyridine, **3r**. M.p. 122-124°C. Cryst. solvent: Ethanol. Yield: 23%. ^{1}H-NMR (δ, ppm): 8.565 (dd, 1H, J = 6.72 Hz, J = 1.08 Hz), 8.536 (s, 1H), 8.302 (d, 2H, J = 7.58 Hz), 7.617 (d, 2H, J = 8.72 Hz), 7. 585 (d, 1H, J = 7.57 Hz), 7,552 (m, 2H), 7.381 (t, 1H, J = 7.82 Hz), 7.030 (t, 1H, J = 6.96 Hz), 6.923 (dd, 1H, J = 8.26 Hz, J = 1.68 Hz), 3.838 (s, 3H). ^{13}C-NMR (δ, ppm): 160.11, 144.65, 143.48, 135.63, 135.31, 133.28, 130.81,128.80, 127.08, 126.95, 123.62, 118.60, 113.71, 113.02, 111.68, 110.59, 55.59. HRMS (ESI, m/z): calcd for $C_{20}H_{15}ClN_2O$ 334.08674, found $[M+H]^+$ 335.09457.

2-(3-Methoxyphenyl)-8-(3,4,5-trimethoxyphenyl)imidazo[1,2-*a*]pyridine, **3s**. M.p. 119-121°C. Cryst. solvent: Methanol. Yield: 20%. ^{1}H-NMR (δ, ppm): 8.540-8.538 (m, 2H), 7.744 (s, 2H), 7.643-7.573 (m, 3H), 7.395 (t, 1H, J = 7.92 Hz), 7.030 (t, 1H, J = 6.88 Hz), 7.390 (dd, 1H, J = 8.16 Hz, J = 1.88 Hz), 3.924 (s, 6H), 3.832 (s, 3H), 3.830 (s, 3H). ^{13}C-NMR (δ, ppm): 160.10, 153.04, 144.34, 143.66, 138.05, 135.75, 131.68, 130.34, 127.87, 126.39, 123.06, 118.35, 114.17, 113.00, 110.88, 110.39, 106.63, 60,58 56.34. HRMS (ESI, m/z): calcd for $C_{23}H_{22}N_2O_4$ 390.15741, found $[M+H]^+$ 391.16523.

8-([1,1'-Biphenyl]-4-yl)-2-(3-methoxyphenyl)imidazo[1,2-*a*]pyridine, **3t.** M.p. 165°C. Cryst. solvent: Ethanol. Yield: 37%. ^{1}H-NMR (δ, ppm): 8.563 (d, 1H, J = 5.76 Hz), 8.543 (s, 1H), 8.372 (d, 2H, J = 8.44 Hz), 7.860 (d, 2H, J = 8.44 Hz), 7.800 (d, 2H, J = 7.25 Hz), 7.616-7.579 (m, 3H), 7.520 (t, 3H, J = 7.48 Hz), 7.431-7.370 (m, 2H), 7.047 (t, 1H, J = 6.92 Hz), 6.927 (dd, 1H, J = 8.04 Hz, J = 0.72 Hz), 3,843 (s, 3H). ^{13}C-NMR (δ, ppm): 159.96, 145.20, 143.99, 142,68, 141.18, 140.86, 139.61, 135.08, 129.65, 129.40, 128.80, 127.39, 127.23, 127.17, 123.29, 118.86, 113.85, 112.96, 111.76, 109.01, 55.42. HRMS (ESI, m/z): calcd for $C_{26}H_{20}N_2O$ 376.15701, found $[M+H]^+$ 377.16484.

Experimental procedures of biochemical and cell viability assays.

Expression and purification of human ALDHs. Large amounts of pure recombinant human ALDH1A1, ALDH1A2 and ALDH1A3 isoenzymes were obtained as previously described.[1] Briefly, induced cells, collected by centrifugation, were re-suspended in an appropriate volume of lysis buffer (50 mM Na_2HPO_4, 300 mM NaCl, 1 mM β-mercaptoethanol, 20 mM imidazole, pH 7.5) added of benzonase nuclease (250 U/μL) and Protease inhibitor cocktail from SIGMA. After ultracentrifugation, the clarified sample was purified by a His-tag affinity chromatography followed by size-exclusion chromatography, using an AKTA FPLC system, at 4 °C. To evaluate the purity and homogeneity of the protein, after each purification step, eluted fractions were analyzed by SDS-PAGE and the protein quantification was always determined by Bradford protein assay. This procedure allowed us to obtain about 20 mg of pure and active human ALDH1A3, ALDH1A2 and ALDH1A1 used for all crystallization trials and kinetic analyses.

Medium-throughput screening to determine the inhibitory efficacy of imidazo[1,2-*a*]pyridine derivatives. A medium throughput screening campaign was performed for enzymes ALDH1A3, ALDH1A1 and ALDH1A2, using the previously reported continuous spectrometric assay for ALDH1A3 optimized to suit Greiner Bio-One® 96-wells UV-Transparent Microplate[2] and following a selection strategy for hit identification of various chemical nature that proved successful in other campaigns carried out in our laboratory against various targets.[3-5] Tests were carried out by using 200 μl of reaction mixture, containing 20 mM Tris HCl pH 8.0, 1 mM β-mercaptoethanol, 150 mM KCl, 15% DMSO, 500 μM NAD^+ and 2,6 μM of pure recombinant isoenzymes. Reactions were started by the addition of 20 mM acetaldehyde. Change in absorbance at 340 nm (ε_{NADH}=6220 M^{-1} cm^{-1}) was monitored for 30 min in a Tecan® Sunrise at 25 °C. The activity of each enzymes was tested in the presence of each synthesized compound at 25 μM concentration, in triplicates.

Enzyme kinetic analysis to calculate IC_{50} values of the most promising compound. Selected compounds showing the best inhibitory activity at 25 μM were further investigated for their inhibitory efficacy, to calculate their IC_{50} values. The enzymatic inhibition assays were performed in a total volume of 200 μl of 20 mM Tris HCl pH 8.0, 1 mM β-mercaptoethanol, 150 mM KCl, 15% DMSO, 500 μM NAD^+, 2,6 μM of pure recombinant isoenzymes in the presence of different inhibitors concentrations from 100 μM to 0.78125 μM and pre-incubated for 5 minutes. Reactions were started by the addition of 20 mM acetaldehyde. The kinetic parameters were determined by fitting the measured data to a Michaelis-Menten curve,[6] by using SigmaPlot.[7]

Enzyme kinetic analysis to calculate the K_i values of 3c and 3f. The enzymatic inhibition assays were performed in a total volume of 200 μl of 20 mM Tris HCl pH 8.0, 1 mM β-mercaptoethanol, 150 mM KCl, 15% DMSO, 500 μM NAD^+, 2,6 μM of pure recombinant isoenzymes in the presence of different inhibitors concentrations. For the complex **3c**-ALDH1A3 the inhibitor was tested at 50, 25, 10, 5, 2.5, 1 and 0.5 μM. For the complex **3f**-ALDH1A3 the inhibitor was tested at 50, 5, 0.5, 0.05 and 0.005 μM. Reactions were started by the addition of different concentration of acetaldehyde, from 1 mM to 20 mM, after 5 minutes of pre-incubation. The kinetic parameters were determined by fitting the measured data to a Michaelis-Menten curve,[6] by using SigmaPlot.[7]

Cell viability assay. 10 x 10^5 U87-MG human glioblastoma cells were plated in Eagle's Minimum Essential Medium (MEM) and treated for 72 hours with the test compound, **3c**, solubilized in DMSO (in a final concentration of 10%). Cells viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay as described elsewhere.[8]

Experimental procedures of crystallization studies and structure determination.

Crystals of ALDH1A3 in complex with **3c** were obtained by using the vapour-diffusion technique in sitting drop and applying a spare-matrix-based strategy with a crystallization robot (Oryx4, Douglas Instruments). The best crystals were grown by mixing 0.5 μl of protein solution at a concentration of 8 mg/mL, pre-incubated with 1 mM NAD^+ and 300 μM MF13, with an equal volume of a reservoir solution containing 2.4 M sodium malonate, pH 7.0, and equilibrated against 50 μl of the reservoir solution, at 20 °C in about 30 days. For X-ray data collection, crystals were quickly equilibrated in a solution containing the crystallization buffer and 12.5% glycerol as cryo-protectant and flash frozen at 100 K in liquid nitrogen. Data up to 3.2 Å resolution were collected at the beamline ID29 European Synchrotron Radiation Facility (ESRF, Grenoble, France). Analysis of the diffraction data set allowed us to assign the crystal to the orthorhombic $P2_122_1$ space group with cell dimensions of a = 81.56 Å, b = 89.36 Å, c = 159.04 Å and $\alpha = \beta = \gamma = 90°$, containing four molecules per asymmetric unit with a corresponding solvent content of 52.3%. Data were processed using the program package XDS[9] and the CCP4 suite of programs[10] was used for scaling. The structure determination of ALDH1A3 was carried out by means of the molecular replacement technique using the coordinates of the tetramer of human ALDH1A3 as the search model (Protein Data Bank ID code 5FHZ[2]). PHASER[11] was used to automatically determine the ALDH1A3 structure in complex with **3c**. The initial model was subjected to iterative cycles of crystallographic refinement with the programs REFMAC5[12] and PHENIX.REFINE,[13] alternated with manual graphic session for model building using the program Coot.[14] 5% of randomly chosen reflections were excluded from refinement of the structure and used for the Free R factor calculation.[15] The program ARP/wARP[16] was used for adding solvent molecules. Refinement was continued until convergence to R-factor and free R-factor values of 0.1962 and 0.2601 respectively, with ideal geometry. Data collection and refinement statistics are given in Table 1-SI (Supporting Information). The atomic coordinates and structure factors of human ALDH1A3 in

complex with GA11 have been deposited with the Protein Data Bank (www.rcsb.org) with the accession code 6TRY. Figures were generated using the program PyMOL.[17]

Experimental procedures of computational studies.

Docking calculations were performed with the Gold program,[18] using the CHEMPLP fitness function. The crystallographic structure of ALDH1A3 in complex with **3c** molecule was used as rigid receptor in molecular docking simulations. The structure of **3f** was sketched in 2D format using Picto (OpenEye) version 4.4.0.4, converted in 3D coordinates by OMEGA (OpenEye) version 3.1.0.3[19] and then energy minimized using the MMFF94S force field.[20] GOLD default parameters were used, while docking efficiency was increase to the maximum value.

MD simulations were carried out on docking complexes using Amber18.[21] The ff14SB force field[22] was used to parameterize the protein and the GAFF force field[23] was used for compound **3f**. Docking complexes were solvated in a rectilinear box of explicit TIP3P water molecules. First, energy minimization was carried out only on water molecules for 5000 steps in which the first 1500 used the steepest descent algorithm (SDA), and the last 3500 the conjugate gradient algorithm (CGA). Then, whole systems were energy minimized for 10000 steps in which the first 1500 with the SDA, and the last 8500 with the CGA. Temperature was gradually raised at constant volume (NVT ensemble) from 0 K to 300 K over 1 ns by the Langevin thermostat. Density equilibration was carried out at constant pressure (NPT ensemble) by the Beredensen barostat, for 1 ns with periodic boundaries conditions. Finally, after an equilibration of 50 ns (NPT ensemble), MD trajectories were produced for of 300 ns (NPT ensemble). Then, the Cpptraj software was used to analyze the MD trajectories.[24]

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