

Supporting Information

HIV-1 Integrase Inhibitor-Inspired Antibacterials Targeting Isoprenoid Biosynthesis

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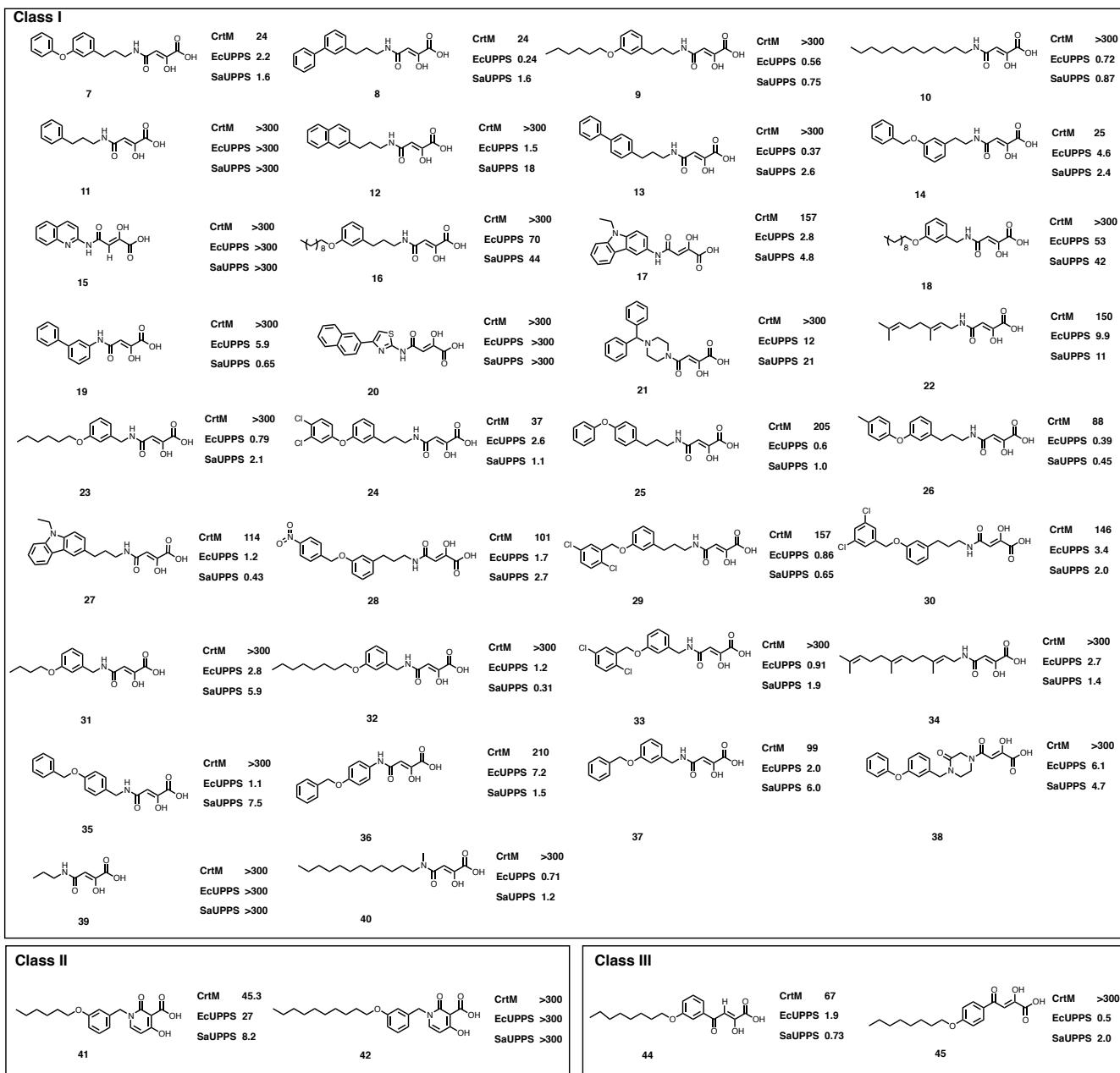


Figure S1. Chemical structures of the screening library compounds and their inhibition for CrtM, *E. coli* UPPS (EcUPPS) and *S. aureus* UPPS (SaUPPS), IC₅₀ values, μM.

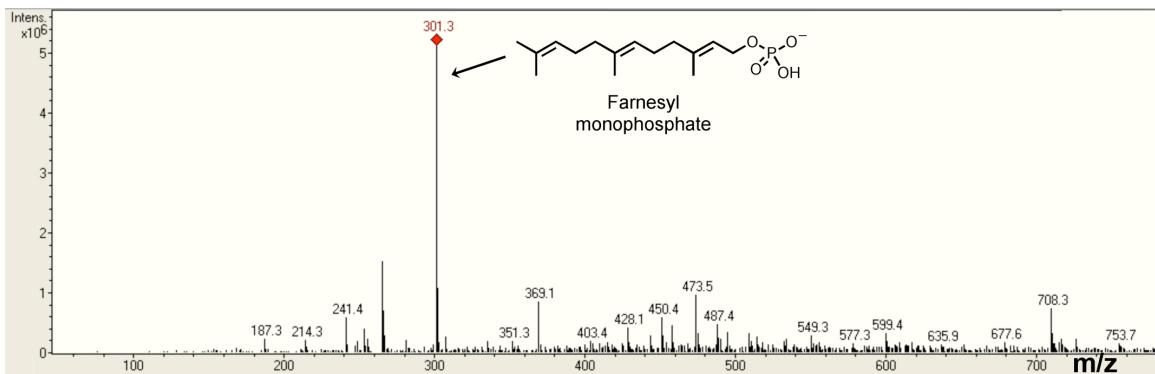


Figure S2. Mass spectrum of farnesyl monophosphate that co-purified with CrtM. The sample was obtained by dissolving CrtM+7+FMP crystals and injecting the solution into an LC-MS instrument.

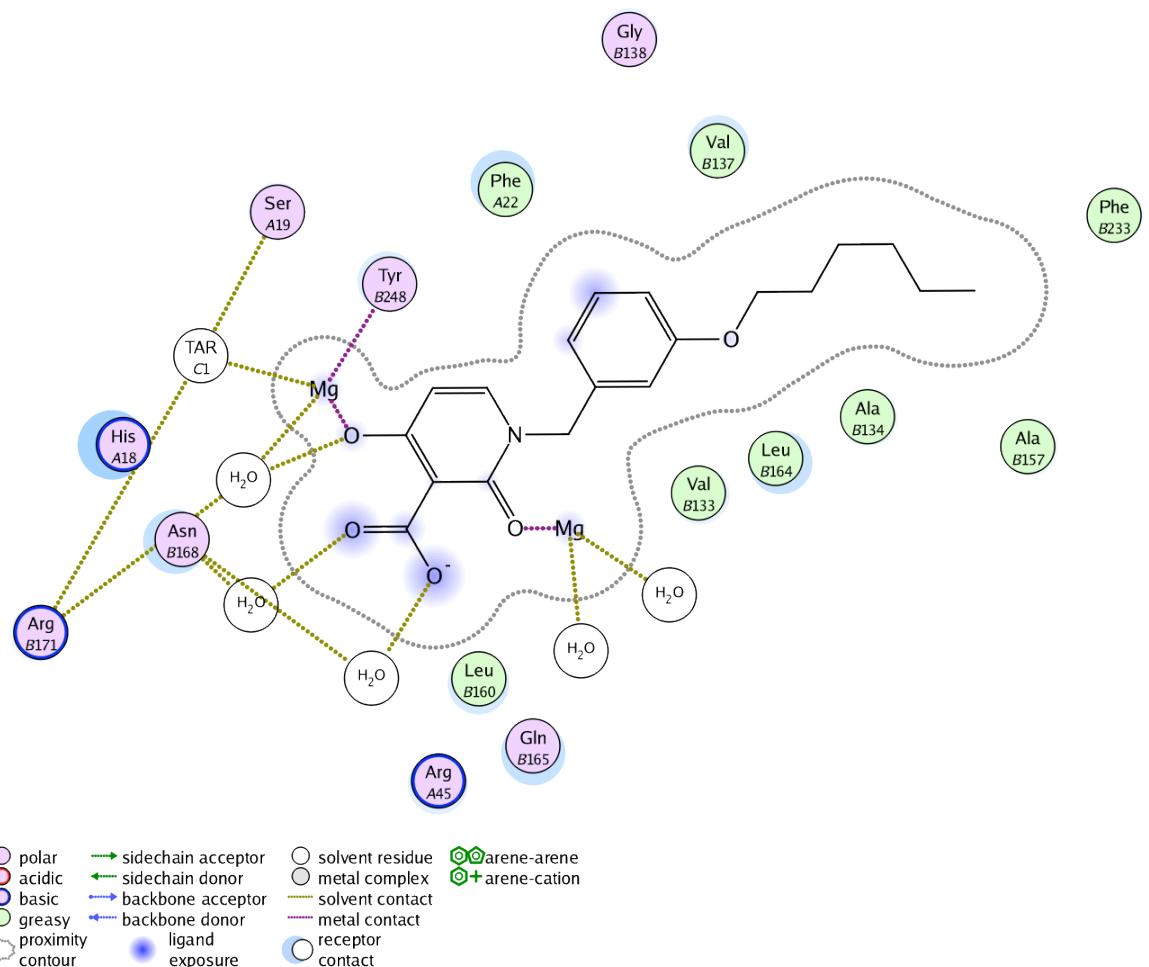


Figure S3. Ligand-protein interactions for **41** binding to CrtM.

Table S1. Data collection and refinement statistics of CrtM-inhibitor and UPPS-inhibitor complexes.

	7 (Soaking) (3P00)	41 (Soaking) (3PAI)	9 (Soaking) (3TH8)
Radiation source	APS 21-ID-F	APS 21-ID-G	APS 21-ID-F
Wavelength (Å)	0.97857	0.97857	0.97872
Space group	P3 ₂ 1	P3 ₂ 1	P2 ₁ 2 ₁ 2 ₁
a(Å)	80.712	80.194	62.603
b(Å)	80.712	80.194	68.862
c (Å)	90.864	91.333	112.297
Resolution (Å)	50.00-2.30 (2.34-2.30)	50.00-1.91 (1.94-1.91)	50.00-2.12 (2.16-2.12)
No. of reflections	29147 (1422)	26704 (1132)	27971 (1389)
Completeness (%)	98.8 (95.4)	98.7 (87.0)	98.2 (100.0)
Redundancy	3.2 (3.0)	5.5 (3.9)	6.0 (6.2)
R _{merge} (%)	8.3 (33.3)	7.2 (30.5)	9.8 (60.4)
I/s(I)	18.6 (3.0)	39.5 (3.9)	24.4 (2.3)
Refinement			
Resolution (Å)	50.00-2.06	30.00-1.98	41.80-2.11
No. of reflections	14844 (779)	23039 (1357)	29046 (1400)
R _{work} (%)	19.9 (23.6)	20.2 (22.8)	24.9 (34.9)
R _{free} (%)	26.67 (25.6)	23.8 (29.5)	30.9 (37.6)
Geometry deviations			
Bond lengths (Å)	0.005	0.007	0.021
Bond angles (°)	0.62	1.01	1.791
Mean B-values (Å ²) / number of non-H atoms			
All refined atoms	33.2 / 2547	32.8 / 2590	36.0 / 3401
Ligand atoms	53.3 / 55	43.6 / 21	51.7 / 25
Mg ions	51.6 / 2	43.3 / 3	
Water molecules	36.4 / 123	35.2 / 178	37.0 / 63
Ramachandran plot (%)			
Most favored	97.8	97.1	91.9
Additionally allowed	1.8	2.5	7.6
Generously allowed	0.4	0.4	0.5

Experimental Section

Enzyme expression and purification. *S. aureus* CrtM was expressed and purified as described previously.¹ Expression and purification of *E. coli* UPPS and *S. aureus* UPPS were also carried out as described previously.²

CrtM inhibition. The *S. aureus* CrtM inhibition assay was carried out as described in our previous work.^{1a}

UPPSi. The *E. coli* UPPS and *S. aureus* UPPS inhibition assays were carried out as described.²

X-ray crystallography. Native CrtM crystals (space group *P*3₂1) were grown by using the hanging-drop method by mixing equal amounts of reservoir with 0.2-1.0 M potassium sodium tartrate, at room temperature. Inhibitor bound crystals were obtained by either soaking the native crystals with 1 mM ligand for 1-4 hours, or incubating protein-ligand (1 mM) mixtures at RT for 1-4 hours, then adding the reservoir solution. All CrtM crystals belonged to the *P*3₂1 space group and had similar lattice parameters.

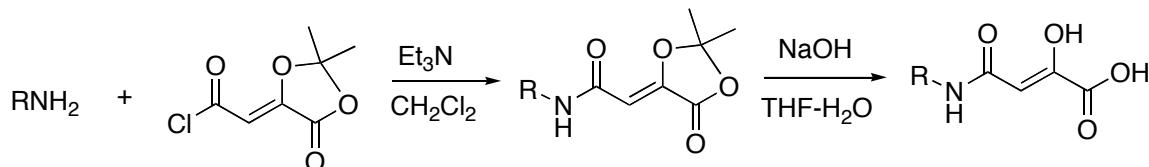
Native *E. coli* UPPS crystals for soaking were obtained by using the hanging-drop method (Hampton Research, Laguna Niguel, CA) by mixing 1 μ L of UPPS protein solution (14 mg/ml UPPS in 50 mM HEPES, pH 7.5) with 1 μ L of mother liquor (50 mM HEPES, pH 7.5, 5% PEG 2-4K) and then equilibrating with 500 μ L mother liquor at room temperature. Crystals grew to 0.3×0.3×0.2 mm in 2 days and were then soaked in a cryoprotectant solution (50 mM HEPES, pH 7.5, 30% EG 5% PEG 35K) containing 2.5-5 mM inhibitor for 1 day.

Diffraction data were collected at sector 21 of the Advanced Photon Source, Argonne National Laboratory. The data were indexed, integrated and scaled by using the HKL2000 program package.³ Structures were determined by molecular replacement with the Phaser program,⁴ using apo CrtM (PDB ID 2ZCP, minus ligands) as a template. The structure of the UPPS-complex was determined by using a model prepared from the UPPS/BPH-629 complex structure (PDB ID 2E98) with ligands and solvent removed. Further model building, ligand preparation, and refinement employed Coot,⁵ ProDRG server,⁶ and Refmac in CCP4,⁷ respectively. All figures were prepared using PyMol (<http://www.pymol.org>).

Cell growth inhibition. The growth of *S. aureus* (USA300 strain) and determination of MIC were as described previously.⁸

Synthesis of library compounds. All reagents used were purchased from Aldrich or Alfa Aesar. The purity of all compounds was routinely monitored by using ¹H NMR spectroscopy on Varian (Palo Alto, CA) Unity spectrometers and by micro-chemical analysis or HRMS.

General procedure for the synthesis of Class I compounds: To a solution of the appropriate amine (0.5 mmol) in dry CH_2Cl_2 (2 mL) was added dry triethylamine (1 mmol) and (*Z*)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-ylidene)acetyl chloride at 0 °C. The mixture was stirred for 3 h and then washed with water (4 mL). The organic layer was dried over Na_2SO_4 , concentrated and subjected to flash chromatography affording the ester, which upon saponification (NaOH, 4 equivalents; 4:1 THF/ H_2O , 10 mL) and acidification gave the final product ~ 60% overall yield.



(*Z*)-2-hydroxy-4-oxo-4-((3-(3-phenoxyphenyl)propyl)amino)but-2-enoic acid (7).

^1H NMR (400 MHz, DMSO-d6) δ 7.34-6.73 (m, 9H), 3.24 (s, 2H), 2.98 (m, 2H), 2.53 (m, 2H), 1.63 (m, 2H). HRMS [M + H]⁺ calcd. for $\text{C}_{19}\text{H}_{20}\text{NO}_5$ 342.1341, found 342.1348.

(*Z*)-4-((3-([1,1'-biphenyl]-3-yl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (8).

^1H NMR (400 MHz, CDCl_3) δ 7.58 (d, $J = 5.6$ Hz, 2H), 7.45-7.33 (m, 6H), 7.17 (d, $J = 6.0$ Hz, 1H), 5.93 (s, 1H), 5.74 (broad, 1H), 3.40 (dd, $J = 5.2, 10.8$ Hz, 2H), 2.74 (t, $J = 6.4$ Hz, 2H), 1.95 (t, $J = 6.0$ Hz, 2H). Anal. Calcd. for $\text{C}_{19}\text{H}_{19}\text{NO}_4$: C, 70.14; H, 5.89; N, 4.31. Found: C, 69.76; H, 5.83; N, 4.40.

(*Z*)-4-((3-(3-(hexyloxy)phenyl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (9).

^1H NMR (400 MHz, CDCl_3) δ 7.18 (m, 1H), 6.72 (m, 3H), 5.89 (s, 1H), 5.65 (s, 1H), 3.92 (t, $J = 6.4$ Hz, 2H), 3.36 (m, 2H), 2.63 (m, 2H), 1.88 (m, 2H), 1.75 (m, 2H), 1.43 (m, 2H), 1.32 (m, 4H), 0.88 (t, $J = 6.4$ Hz, 3H). HRMS [M + H]⁺ calcd. for $\text{C}_{19}\text{H}_{28}\text{NO}_5$ 350.1967, found 350.1970.

(*Z*)-4-(dodecylamino)-2-hydroxy-4-oxobut-2-enoic acid (10).

^1H NMR (400 MHz, CDCl_3) δ 6.16 (broad, 1H), 5.90 (s, 1H), 3.27 (m, 2H), 1.49 (m, 2H), 1.21 (m, 18H), 0.84 (t, $J = 6.8$ Hz, 3H). HRMS [M + H]⁺ calcd. for $\text{C}_{16}\text{H}_{30}\text{NO}_4$ 300.2175, found 300.2172.

(*Z*)-2-hydroxy-4-oxo-4-((3-phenylpropyl)amino)but-2-enoic acid (11).

^1H NMR (400 MHz, DMSO-d6) δ 8.54 (t, $J = 5.6$ Hz, 1H), 7.27-7.13 (m, 5H), 5.95 (s, 1H), 3.12 (dd, $J = 6.4, 12.8$ Hz, 2H), 2.56 (t, $J = 8.0$ Hz, 2H), 1.71 (m, 2H). Anal. Calcd. for $\text{C}_{13}\text{H}_{15}\text{NO}_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.61; H, 5.99; N, 5.65.

(Z)-2-hydroxy-4-((3-(naphthalen-2-yl)propyl)amino)-4-oxobut-2-enoic acid (12).

¹H NMR (400 MHz, DMSO-d₆) δ 8.58 (broad, 1H), 7.80 (m, 3H), 7.68 (s, 1H), 7.46-7.36 (m, 3H), 5.96 (s, 1H), 3.18 (dd, *J* = 6.8, 12.8 Hz, 2H), 2.74 (t, *J* = 7.6 Hz, 2H), 1.82 (m, 2H). HRMS [M + H]⁺ calcd. for C₁₇H₁₈NO₄ 300.1236, found 300.1231.

(Z)-4-((3-([1,1'-biphenyl]-4-yl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (13).

¹H NMR (400 MHz, DMSO-d₆) δ 8.56 (t, *J* = 6.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.41 (t, *J* = 6.8 Hz, 2H), 7.31 (m, 3H), 5.96 (s, 1H), 3.16 (dd, *J* = 6.0, 12.8 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.76 (m, 2H). Anal. Calcd. for C₁₉H₁₉NO₄: C, 70.14; H, 5.89; N, 4.31. Found: C, 69.80; H, 5.74; N, 4.44.

(Z)-4-((3-(3-(benzyloxy)phenyl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (14).

¹H NMR (400 MHz, DMSO-d₆) 7.40-7.28 (m, 5H), 7.11 (m, 1H), 6.84-6.75 (m, 3H), 5.03 (s, 2H), 3.32 (s, 2H), 2.99 (m, 2H), 2.51 (m, 2H), 1.64 (m, 2H). HRMS [M + H]⁺ calcd. for C₂₀H₂₂NO₅ 356.1498, found 356.1494.

(Z)-2-hydroxy-4-oxo-4-(quinolin-2-ylamino)but-2-enoic acid (15).

¹H NMR (400 MHz, D₂O) δ 7.99 (d, *J* = 9.2 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.55-7.47 (m, 2H), 7.26 (t, *J* = 7.2 Hz, 1H). HRMS [M + H]⁺ calcd. for C₁₃H₁₁N₂O₄ 259.0719, found 259.0724.

(Z)-4-((3-(3-(decyloxy)phenyl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (16).

¹H NMR (400 MHz, CDCl₃) δ 7.19 (m, 1H), 6.72 (m, 3H), 5.90 (s, 1H), 5.62 (broad, 1H), 3.91 (t, *J* = 6.8 Hz, 2H), 3.75 (m, 2H), 2.63 (m, 2H), 1.88 (m, 2H), 1.75 (m, 2H), 1.82-1.25 (m, 14H), 0.86 (t, *J* = 6.8 Hz, 3H). Anal. Calcd. for C₂₃H₃₅NO₅: C, 68.12; H, 8.70; N, 3.45. Found: C, 67.98; H, 8.98; N, 3.70.

(Z)-4-((9-ethyl-9H-carbazol-3-yl)amino)-2-hydroxy-4-oxobut-2-enoic acid (17).

¹H NMR (400 MHz, D₂O) δ 8.02 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.38-7.28 (m, 4H), 7.07 (t, *J* = 6.8 Hz, 1H), 4.18 (dd, *J* = 7.2, 14.4 Hz, 2H), 1.13 (t, *J* = 7.2 Hz, 3H). HRMS [M + H]⁺ calcd. for C₁₈H₁₇N₂O₄ 325.1188, found 325.1195.

(Z)-4-((3-(decyloxy)benzyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (18).

¹H NMR (400 MHz, CD₃Cl) δ 7.22 (m, 1H), 6.79 (m, 3H), 6.06 (s, 1H), 4.45 (d, *J* = 5.2 Hz, 2H), 3.90 (m,

2H), 1.73 (m, 2H), 1.41-1.19 (m, 14H), 0.85 (t, J = 6.8 Hz, 3H).

HRMS [M + H]⁺ calcd. for C₂₁H₃₂NO₅ 378.2280, found 378.2275.

(Z)-4-([1,1'-biphenyl]-3-ylamino)-2-hydroxy-4-oxobut-2-enoic acid (19).

¹H NMR (400 MHz, CDCl₃) δ 9.26 (s, 1H), 7.84 (s, 1H), 7.54-7.53 (m, 4H), 7.40-7.28 (m, 5H), 6.22 (s, 1H). HRMS [M + H]⁺ calcd. for C₁₆H₁₄NO₄ 284.0923, found 284.0923.

(Z)-2-hydroxy-4-((4-(naphthalen-2-yl)thiazol-2-yl)amino)-4-oxobut-2-enoic acid (20).

¹H NMR (400 MHz, DMSO-d6) δ 9.20 (broad, 2H), 8.06-7.97 (m, 3H), 7.65-7.55 (m, 4H), 7.02 (s, 1H). HRMS [M + H]⁺ calcd. for C₁₇H₁₃N₂O₄S 341.0596, found 341.0598.

(Z)-4-(4-benzhydrylpiperazin-1-yl)-2-hydroxy-4-oxobut-2-enoic acid (21).

¹H NMR (400 MHz, D₂O) δ 7.32 (d, J = 6.0 Hz, 4H), 7.20 (t, J = 5.6 Hz, 4H), 7.11 (t, J = 5.6 Hz, 2H), 3.42 (m, 2H), 3.28 (m, 2H), 2.29 (m, 4H). HRMS [M + H]⁺ calcd. for C₂₁H₂₃N₂O₄ 367.1658, found 367.1667.

(Z)-4-((E)-3,7-dimethylocta-2,6-dien-1-yl)amino)-2-hydroxy-4-oxobut-2-enoic acid (22).

¹H NMR (400 MHz, CDCl₃) δ 5.95 (s, 1H), 5.64 (broad, 1H), 5.17 (m, 1H), 5.04 (m, 1H), 3.92 (t, J = 6.0 Hz, 2H), 2.06 (m, 4H), 1.67 (s, 6H), 1.58 (s, 3H). HRMS [M + H]⁺ calcd. for C₁₄H₂₂NO₄ 268.1549, found 268.1540.

(Z)-4-((3-(hexyloxy)benzyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (23).

¹H NMR (400 MHz, DMSO-d6) δ 8.92 (t, J = 5.6 Hz, 1H), 7.20 (m, 1H), 6.78 (m, 3H), 5.99 (s, 1H), 4.30 (d, J = 6.0 Hz, 2H), 3.89 (m, 2H), 1.65 (t, J = 8.0 Hz, 2H), 1.35-1.25 (m, 6H), 0.83 (t, J = 6.8 Hz, 3H). HRMS [M + H]⁺ calcd. for C₁₇H₂₄NO₅ 322.1654, found 322.1658.

(Z)-4-((3-(3,4-dichlorophenoxy)phenyl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (24).

¹H NMR (400 MHz, CDCl₃) δ 7.35-7.2 (m, 2H), 7.02-6.78 (m, 5H), 5.92 (broad, 1H), 5.90 (s, 1H), 3.36 (m, 2H), 2.62 (m, 2H), 1.83 (m, 2H). HRMS [M + H]⁺ calcd. for C₁₉H₁₈Cl₂NO₅ 410.0562, found 410.0563.

(Z)-2-hydroxy-4-oxo-4-((3-(4-phenoxyphenyl)propyl)amino)but-2-enoic acid (25).

¹H NMR (400 MHz, DMSO-d6) δ 8.56 (t, J = 4.4 Hz, 1H), 7.35 (m, 2H), 7.20 (d, J = 7.2 Hz, 2H), 7.09 (t, J = 6.0 Hz, 1H), 6.95 (d, J = 6.4 Hz, 2H), 6.91 (d, J = 7.2 Hz, 2H), 5.97 (s, 1H), 3.16 (dd, J = 5.6, 10.4 Hz, 2H), 2.57 (t, J = 7.8 Hz, 2H), 1.73 (m, 2H). HRMS [M + H]⁺ calcd. for C₁₉H₂₀NO₅ 342.1341, found

(Z)-2-hydroxy-4-oxo-4-((3-(3-(p-tolyloxy)phenyl)propyl)amino)but-2-enoic acid (26).

¹H NMR (400 MHz, CDCl₃) δ 7.24 (t, *J* = 6.0 Hz, 1H), 7.14 (d, *J* = 6.8 Hz, 2H), 6.91 (d, *J* = 6.8 Hz, 2H), 6.88 (m, 1H), 6.81 (s, 2H), 5.95 (s, 1H), 5.73 (broad, 1H), 3.38 (m, 2H), 2.64 (t, *J* = 6.0 Hz, 2H), 2.34 (s, 3H), 1.90 (m, 2H). HRMS [M + H]⁺ calcd. for C₂₀H₂₂NO₅ 356.1498, found 356.1494.

(Z)-4-((3-(9-ethyl-9H-carbazol-3-yl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (27).

¹H NMR (400 MHz, DMSO-d6) δ 8.58 (t, *J* = 5.2 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.93 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.12 (t, *J* = 7.2 Hz, 1H), 5.97 (s, 2H), 4.36 (d, *J* = 7.2 Hz, 2H), 3.18 (m, 2H), 2.73 (t, *J* = 7.2 Hz, 2H), 1.81 (t, *J* = 7.2 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H). HRMS [M + H]⁺ calcd. for C₂₁H₂₃N₂O₄ 367.1658, found 367.1660.

(Z)-2-hydroxy-4-((3-(3-((4-nitrobenzyl)oxy)phenyl)propyl)amino)-4-oxobut-2-enoic acid (28).

¹H NMR (400 MHz, DMSO-d6) δ 8.53 (t, *J* = 7.6 Hz, 1H), 8.22 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.16 (dd, *J* = 3.6, 8.0 Hz, 1H), 6.86-6.77 (m, 2H), 5.94 (s, 1H), 5.22 (s, 2H), 3.15-3.08 (m, 2H), 2.52 (dd, *J* = 7.2, 14.8 Hz, 2H), 1.72-1.63 (m, 2H). HRMS [M + H]⁺ calcd. for C₂₀H₂₁N₂O₇ 401.1349, found 401.1346.

(Z)-4-((3-(3-((2,5-dichlorobenzyl)oxy)phenyl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (29).

¹H NMR (400 MHz, DMSO-d6) δ 8.52 (s, 1H), 7.52 (s, 1H), 7.53-7.47 (m, 3H), 7.38 (s, 1H), 7.18-7.15 (m, 1H), 6.84-6.77 (m, 2H), 5.94 (s, 1H), 5.07 (s, 2H), 3.14-3.09 (m, 2H), 2.52 (dd, *J* = 6.8, 14.0 Hz, 2H), 1.70 (t, *J* = 6.4 Hz, 2H). HRMS [M + H]⁺ calcd. for C₂₀H₂₀Cl₂NO₅ 424.0719, found 424.0723.

(Z)-4-((3-(3-((3,5-dichlorobenzyl)oxy)phenyl)propyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (30).

¹H NMR (400 MHz, DMSO-d6) δ 8.52 (s, 1H), 7.52 (s, 1H), 7.53-7.47 (m, 3H), 7.38 (s, 1H), 7.18-7.15 (m, 1H), 6.84-6.77 (m, 2H), 5.94 (s, 1H), 5.07 (s, 2H), 3.14-3.09 (m, 2H), 2.52 (dd, *J* = 6.8, 14.0 Hz, 2H), 1.70 (t, *J* = 6.4 Hz, 2H). HRMS [M + H]⁺ calcd. for C₂₀H₂₀Cl₂NO₅ 424.0719, found 424.0723.

(Z)-4-((3-butoxybenzyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (31).

¹H NMR (400 MHz, DMSO-d6) δ 8.90 (broad, 1H), 7.18 (m, 1H), 6.76 (m, 3H), 6.00 (s, 1H), 4.28 (d, *J* = 6.0 Hz, 2H), 3.85 (m, 2H), 1.62 (m, 2H), 1.38 (m, 2H), 0.82 (t, *J* = 6.8 Hz, 3H). HRMS [M + H]⁺ calcd. for C₁₅H₂₀NO₅ 294.1341, found 294.1345.

(Z)-2-hydroxy-4-((3-(octyloxy)benzyl)amino)-4-oxobut-2-enoic acid (32).

¹H NMR (400 MHz, DMSO-d₆) δ 7.15 (m, 1H), 6.76 (m, 3H), 4.22 (d, *J* = 6.0 Hz, 2H), 3.91 (m, 2H), 3.42 (s, 2H), 1.66 (m, 2H), 1.34-1.23 (m, 10H). 0.83 (t, *J* = 6.4 Hz, 3H). HRMS [M + H]⁺ calcd. for C₁₉H₂₈NO₅ 350.1967, found 350.1975.

(Z)-4-((3-((2,5-dichlorobenzyl)oxy)benzyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (33).

¹H NMR (400 MHz, DMSO-d₆) δ 13.50(broad, 1H), 8.98 (s, 1H), 7.63-7.24 (m, 4H), 6.88 (m, 3H), 6.00 (s, 1H), 5.10 (s, 2H), 4.34 (d, *J* = 5.6 Hz, 2H). HRMS [M + H]⁺ calcd. for C₁₈H₁₆Cl₂NO₅ 396.0406, found 396.0413.

(Z)-2-hydroxy-4-oxo-4-(((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)amino)but-2-enoic acid (34).

¹H NMR (400 MHz, CDCl₃) δ 6.38 (broad, 1H), 5.94 (s, 1H), 5.18 (dd, *J* = 7.2, 14.8 Hz, 1H), 5.06 (t, *J* = 6.4 Hz, 2H), 3.90 (m, 2H), 2.10-1.95 (m, 8H), 1.65 (s, 6H), 1.57 (s, 6H). HRMS [M + H]⁺ calcd. for C₁₉H₃₀NO₄ 336.2175, found 336.2170.

(Z)-4-((4-(benzyloxy)benzyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (35).

¹H NMR (400 MHz, DMSO-d₆) δ 8.91 (s, 1H), 7.41-7.28 (m, 5H), 7.17 (d, *J* = 8.0 Hz, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 5.98 (s, 1H), 5.06 (s, 2H), 4.26 (d, *J* = 5.6 Hz, 2H). HRMS [M + H]⁺ calcd. for C₁₈H₁₈NO₅ 328.1185, found 328.1181.

(Z)-4-((4-(benzyloxy)phenyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (36).

¹H NMR (400 MHz, DMSO-d₆) δ 7.43-7.07 (m, 8H), 6.75 (dd, *J* = 1.6, 8.4 Hz, 1H), 6.15 (s, 1H), 5.05 (s, 2H). HRMS [M + H]⁺ calcd. for C₁₇H₁₆NO₅ 314.1028, found 314.1030.

(Z)-4-((3-(benzyloxy)benzyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (37).

¹H NMR (400 MHz, DMSO-d₆) δ 7.42-7.27 (m, 5H), 7.20 (m, 1H), 6.92-6.80 (m, 3H), 6.00 (s, 1H), 5.05 (s, 2H), 4.31 (d, *J* = 5.6 Hz, 2H). HRMS [M + H]⁺ calcd. for C₁₈H₁₈NO₅ 328.1185, found 328.1182.

(Z)-2-hydroxy-4-oxo-4-(3-oxo-4-(3-phenoxybenzyl)piperazin-1-yl)but-2-enoic acid (38).

¹H NMR (400 MHz, DMSO-d₆) δ 7.38-7.30 (m, 3H), 7.11 (t, *J* = 7.2 Hz, 1H), 6.98 (s, 2H), 6.97 (s, 1H), 6.89-6.84 (m, 2H), 4.50 (s, 2H), 4.13 (s, 1H), 4.04 (s, 1H), 3.63 (t, *J* = 4.2 Hz, 2H), 3.44 (d, *J* = 3.2 Hz,

2H), 3.19 (m, 2H). HRMS [M + H]⁺ calcd. for C₂₁H₂₁N₂O₆ 397.1400, found 397.1408.

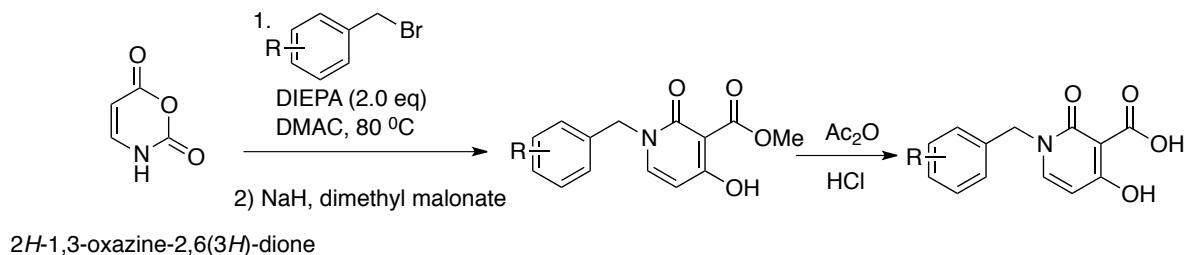
(Z)-2-hydroxy-4-oxo-4-(propylamino)but-2-enoic acid (39).

¹H NMR (400 MHz, CDCl₃) δ 5.96 (s, 1H), 5.63 (broad, 1H), 3.31 (m, 2H), 1.58 (m, 2H), 0.94 (m, 3H). HRMS [M + H]⁺ calcd. for C₇H₁₂NO₄ 174.0766, found 174.0768.

(Z)-4-(dodecyl(methyl)amino)-2-hydroxy-4-oxobut-2-enoic acid (40).

¹H NMR (400 MHz, CDCl₃) δ 6.28 (s, 1H), 3.45 (m, 2H), 3.03 (m, 3H), 1.59 (m, 2H), 1.26 (m, 18H), 0.88 (t, J = 6.8 Hz, 3H). HRMS [M + H]⁺ calcd. for C₁₇H₃₂NO₄ 314.2331, found 314.2327.

41 and **42** were prepared using a similar protocol⁹ starting from 2H-1,3-oxazine-2,6(3H)-dione.



1-(3-(hexyloxy)benzyl)-1,2-dihydro-4-hydroxy-2-oxypyridine-3-carboxylic acid (41).

¹H NMR (400 MHz, D₂O) δ 7.11 (t, J = 8 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 8.4 Hz, 1H), 6.61 (s, 1H), 6.59 (s, 1H), 5.68 (d, J = 7.8 Hz, 1H), 4.79 (s, 2H), 3.82 (t, J = 6.8 Hz, 2H), 1.68-1.49 (m, 2H), 1.18-1.06 (m, 6H), 0.63 (t, J = 6.8 Hz, 3H). HRMS [M + H]⁺ calcd. for C₁₇H₃₂NO₅ 314.2331, found 314.2327. HRMS [M + H]⁺ calcd. for C₁₉H₂₄NO₅ 346.1654, found 346.1649.

1-(3-(decyloxy)benzyl)-1,2-dihydro-4-hydroxy-2-oxypyridine-3-carboxylic acid (42)

¹H NMR (400 MHz, D₂O) δ 7.13 (t, J = 8 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.61 (s, 1H), 6.59 (s, 1H), 5.71 (d, J = 7.8 Hz, 1H), 4.67 (s, 2H), 3.8 (t, J = 6.8 Hz, 2H), 1.66-1.51 (m, 2H), 1.18-1.06 (m, 14H), 0.60 (t, J = 6.8 Hz, 3H). HRMS [M + H]⁺ calcd. for C₂₃H₃₁NO₅ 401.2202, found 401.2205.

44, 45 were prepared according to reported procedures.¹⁰

(Z)-4-(3-(decyloxy)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (44).

¹H NMR (400 MHz, CDCl₃) δ 7.58 (d, J = 6.4 Hz, 1H), 7.50 (s, 1H), 7.41 (t, J = 6.4 Hz, 1H), 7.16 (m,

2H), 4.02 (t, J = 4.2 Hz, 2H), 1.80 (m, 2H), 1.50-1.28 (m, 14H), 0.88 (t, J = 6.0 Hz, 3H). HRMS [M + H]⁺ calcd. for C₂₀H₂₉O₅ 349.2015, found 349.2017.

(Z)-2-hydroxy-4-(4-(octyloxy)phenyl)-4-oxobut-2-enoic acid (45).

¹H NMR (400 MHz, CDCl₃) δ 7.96 (d, J = 8.8 Hz, 2H), 7.07 (s, 1H), 6.95 (d, J = 8.8 Hz, 2H), 4.02 (t, J = 6.4 Hz, 2H), 1.79 (m, 2H), 1.44 (m, 2H), 1.29 (m, 10H), 0.86 (t, J = 7.2 Hz, 3H). HRMS [M + H]⁺ calcd. for C₁₈H₂₅O₅ 321.1702, found 321.1701.

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