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Supporting Information

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Materials and methods

Proteins used in this study

A bacterial expression plasmid encoding His_6-tagged GACKIX domain from mouse CBP (residues 586-672) linked via a polar linker was used to express KIX as previously described.(*2*) Briefly, protein was expressed with 0.1 mM IPTG induction for 3 h at 25 °C with shaking from $pHis_6$-GACKIX transformed Rosetta pLysS cells (Novagen), purified using Ni-NTA beads (Qiagen) and buffer exchanged on PD-10 columns (GE Healthcare) to Storage buffer (10 mM PBS, pH 6.8, 10% glycerol, 0.1% NP-40 and 1 mM DTT). Purity of the protein was verified to be >95% by SDS-PAGE.

To express ^{15}N-labeled GACKIX, a single colony of Rosetta pLysS cells transformed with $pHis_6$-KIX was used to inoculate a LB starter cultures with 0.1 mg/mL ampicillin and 0.034 mg/mL chloramphenicol at 37 °C, grown overnight with shaking. The starter culture was added to 4 L LB with ampicillin and grown at 37 °C until an OD_{600} of 0.7 was reached. Next, cells were pelleted and washed with M9 minimal media (50mM sodium phosphate pH 7.4, 20 mM potassium phosphate, 100 mM sodium chloride, 2 mM magnesium sulfate, 100 mM calcium chloride, 15 mM thiamine, 1.1 g/L $^{15}NH_4Cl$ and 0.4 % glucose), combined and resuspended in 1 L M9 minimal media with ampicillin and BioXpress (Cambridge Isotope Laboratories) added. Cells were allowed to recover with shaking at 37 °C for 1 h before the temperature was reduced to 25 °C and 0.1 M IPTG was added to induce protein expression. After 8 hours, cells were pelleted and frozen at -80 °C. Pellets were lysed in Lysis buffer (50 mM phosphate pH 7.1, 300 mM sodium chloride, 10 mM imidazole) using sonication and purified by Ni-NTA beads. The Ni-NTA eluted protein was further purified by cation exchange FPLC (Source 15S column, GE Healthcare; 0-1 M NaCl gradient in 50 mM PBS buffer). Next, FPLC purified protein

was buffer exchanged using PD-10 columns into 10 mM phosphate pH 6.8, 100 mM NaCl and concentrated to 90-100 μM using 10 KD molecular weight cutoff concentrators (Vivascience) and stored at -80 °C until use. Protein concentration was determined by UV (ε=14440 $M^{-1}cm^{-1}$).

Gal4(1-100), Med15(1-345) and Med15(107-255) were bacterially expressed and purified as previously described.(*3, 4*)

Peptides used in this study

All peptides were synthesized on CLEAR amide resin (Peptides international) using standard HBTU/HOBT/DIEA solid phase peptide synthesis protocols as previously described.(*5*) The TFA-cleaved peptides were precipitated with ether, purified by reverse-phased HPLC using a 0.1% TFA/CH_3CN solvent system and verified by electrospray mass spectrometry.

Fl-MLL19 (FITC-βAla-DCGNILPSDIMDFVLKNTP) used for the screen was synthesized by coupling FITC to βAla-DCGNILPSDIMDFVLKNTP on resin. The concentration of Fl-MLL19 was determined by UV in 10 mM Tris pH 9.0, using ε =77,000 $M^{-1}cm^{-1}$ at 494 nM.

Unlabeled MLL15dm (ILPSDI(nLe)DFILKNTY) was used for competition NMR experiments. The sequence contains a C-terminal tyrosine for quantitation.
The concentration was determined by UV in in 8M Urea, Tris pH 8.0 using ε = 1420 $M^{-1}cm^{-1}$ at 280 nM.

Fl-MLL15dm (FITC-ILPSDI(nLe)DFILKNTY) was used for competitive FP experiments with the depsides and depsidones .

Fl-pKID29 (FITC-AEEA-TDSQKRREILSRRPS(Phos)YRKILNDLSSDAPG) was used for competitive FP experiments with the depsides and depidones.
Unlabeled pKID29 (TDSQKRREILSRRPS(Phos)YRKILNDLSSDAPG) was used for competition NMR experiments.

Fl-Myb (FITC-βAla-KEKRIKELELLLMSTENELKGQQALW) was used for competitive FP experiments with the natural product extracts

Fl-VP2 (Fl-DFDLDMLGDFDLDMLG) and unlabeled VP2 (Biotin-DFDLDMLGD(pBpa)DLDMLG) were used to evaluate the specificity of sekikaic acid

High Throughput Screen

The screen was performed at the Center for Chemical Genomics, University of Michigan. A 50,562 member diverse small molecule library (compounds from Maybridge HitFinder, ChemBridge, ChemDiv, Spectrum, NCI and NIH collection) and a 16,320 natural product extract collection were used to find inhibitors of MLL-GACKIX by fluorescence polarization. The assay was performed in 384-well plates (low volume, NBS, black, 284-

well Corning plates #3676). Each plate was set up such that columns 1-22 contained 5 μL of GACKIX at 20 μM in assay buffer (10 mM PBS pH 6.8, 10% glycerol, 0.01% NonidetP-40, 1 mM DTT), and columns 23-24 contained 5 μL assay buffer. DMSO (200 nL, 2% v/v final) was added with a pin-tool to columns 1 and 2, while columns 3-22 received compounds (final concentration 20 μM for small molecules and 0.3 mg/L for extracts), followed by addition of 5 μL, 50 nM Fl-MLL19 in assay buffer with a liquid handler. Samples were incubated for 30 min before being read on a PheraStar Plus (BMG Labtech) plate reader equipped with the Fl-FP module (485nm/520nm).

Initial hits (inhibition >15% or 2.5 SD for small molecule collection, >20% SD for natural product extracts) were analyzed with consultation of Dr. Paul D. Kirchhoff (Vahlteich Medicinal Chemistry Core, University of Michigan) and Martha Larsen (Center for Chemical Genomics, University of Michigan). Confirmation tests on the initial hits were done in an assay with identical final components, but with a different order of addition. For this, the tracers were added first, followed by compounds, and the plates were read once before addition of protein. The first read was used to determine if any of the compounds quenched the fluorescence of the tracer. As described in the text, no small molecule collection-derived hits proceeded past the secondary confirmation tests.

The confirmatory assay yielded 64 hits from the extracts that were also non-fluorescent. These hits were counter-screened using an assay developed by the Grembecka lab (University of Michigan) with the Fl-MBM1-menin protein-protein interaction (Fl-MBM1 was used at 15 nM while menin was used at 150 nM).(*6*)

All of the subsequent hits from the natural product extracts (22 hits) were found to be from lichen extracts isolated in Costa Rica. Fresh extracts were isolated from Costa Rica (Dr. Giselle Tamayo, National Institute for Biodiversity, Costa Rica) and 13 of those were transferred to the University of Michigan. These extracts were separated into 5 individual HPLC fractions to facilitate identification of the active component(s) see below for details. Each fraction was tested for its ability to inhibit Fl-MLL19-GACKIX at three different concentrations 0.8 mg/mL, 0.08 mg/mL and 0.008 mg/mL (Supporting Figure 2). Next, the most active of these fractions were tested for specificity using three other FP-assays, c-Fl-Myb-GACKIX, Fl-VP2-Med15(1-345) and Fl-DNA-Gal4(1-100). For these, Fl-cMyb and Fl-VP2 were used at 25 nM and the Fl-DNA tracer was used at 10 nM. GACKIX and Med15 were used at 10 μM and Gal4 protein was used at 50 nM while the extract was used at 0.3 mg/mL.

Fluorescent polarization assay – competitive binding experiments

The FP assays were done in triplicate with a final sample volume of 10 μL in a low-volume, non-binding, black, 384-well plate (Corning), and read using a Tecan Genios Pro plate reader with polarized excitation at 485 nm and emission intensity measured through a parallel and perpendicularly polarized 535 nm filter. Fl-MLL15dm – GACKIX inhibition curves were done with GACKIX at 1 μM. Fl-KID29 – GACKIX inhibition curves were done with GACKIX at 2.5 μM. In all cases the tracer was kept constant at 25 nM. The obtained data was plotted in Graphpad Prism 5 and fitted with nonlinear regression using built-in equation "log(inhibitor) vs. response – Variable slope (four

parameters)" from which the IC_{50} was calculated.

Isolation and structure determination of depsides

Microphyllinic acid and 5'-O-demethylmicrophyllinic acid

Collection and Extraction: Approximately 4.28 g of the lichen *Parmotrema mellissii* (Lecanorales, Ascomycota) collected at the Cerro Pedregoso Summit, Guanacaste National Park, Costa Rica (Permit R-CM-INBio-03-2006-OT) on September 2006, afforded 3.70 g of dry material upon drying. The dry powder was extracted with 100 mL ethanol 95% on an ultrasonic bath for 20 minutes and repeated 3 times, to yield 396.5 mg of crude extract.

Fractionation: A solid phase extraction method was employed. A head of approximately 400 mg of crude extract of *P. Mellissii* with Diaion HP-20ss (3:1) were loaded on a column with 4.8 g of Diaion HP-20ss and eluted 5 times with 15 mL elutions of water:ethanol 8:2, 1:1, 2:8, 100% ethanol and isopropanol:dichloromethane 8:2 to afford five fractions. Fraction 3 afforded 74.8 mg of SSCODE 69108, fraction 4 afforded 83.3 mg of SSCODE 69109 and fraction 5 afforded 71.3 mg of SSCODE 69110 which was found to contain microphyllinic acid and 5'-O-demethylmicrophyllinic acid.

Sekikaic Acid

Collection and Extraction: Approximately 1.64 g of the lichen *Dirinaria aegialita* (Lecanorales, Ascomycota) collected nearby the ranger's station in Tempisque, Diriá National Park, Costa Rica (Permit R-CM-INBio-03-2006-OT) on October 2006, afforded 1.57 g upon drying. The dry powder was extracted with 100 mL ethanol 95% on an ultrasonic bath for 20 minutes and repeated 3 times, to yield 367.0 mg of crude extract.

Fractionation: A solid phase extraction method was employed. A head of 360 mg of crude extract of *D. aegialita* with Diaion HP-20ss (3:1) were loaded on a column with 4.5 g of Diaion HP-20ss and eluted 5 times with 15 mL elutions of water:ethanol 8:2, 1:1, 2:8, 100% ethanol and isopropanol:dichloromethane 8:2 to afford five fractions. Fraction 4 afforded 81.1 mg of SSCODE 69127 and as described below this contained sekikaic acid.

HPLC fractions found active in FP assays were characterized by NMR spectroscopy, mass spectrometry and UV-vis to determine the identity of their constituents. Extract 2 contained primarily one compound that was active in the assay. This compound was determined to be sekikaic acid (see Supporting Figures 3 and 4 for NMR and mass spectrometry characterization data). Extract 6 contained two active compounds which were determined to be the depsides microphyllinic acid and 5'-O-demethylmicrophyllinic acid (data not shown). For comparative studies lecanoric acid and lobaric acid were purchased from ChromaDex. A UV spectrum of sekikaic acid was recorded, and ε 260 nm = 12196 $M^{-1}cm^{-1}$ was measured and used for determination of concentrations.

NMR Analysis

1D ^{1}H-NMR studies of sekikaic acid in presence of GACKIX and native GACKIX ligands (Figure 4 in main article)

Unlabeled GACKIX protein was expressed and purified as described above. Spectra were recorded on a Bruker Avance III 600-MHz spectrometer equipped with a cryogenic probe

at 22 ^{o}C. The individual spectra are referenced via d_6-DMSO. For each NMR experiment, 15 μM (final concentration) sekikaic acid (dissolved in d_6-DMSO) was combined with variations of a d_6-DMSO control, 5 μM (final concentration) of GACKIX, 15 μM (final concentration) pKID29 (dissolved in d_6-DMSO) and/or 15 μM (final concentration) MLLdm (dissolved in d_6-DMSO) in 10 mM sodium phosphate pH 6.8, 100 mM NaCl, 10% D_2O (<2% d_6-DMSO in each sample). The samples were incubated at 4 ^{o}C for 30-90 min but were equilibrated to 22 ^{o}C before recording spectra. The Data was analyzed using MestRec/MNova.

^{1}H-^{15}N-HSQC NMR studies of GACKIX in presence of sekikaic acid (Figure 4 in main article)

For these experiments, uniformly ^{15}N-labeled-GACKIX protein was expressed and purified as described above. Uniformly ^{15}N, ^{13}C- labeled-GACKIX was expressed and purified in a similar fashion using M9 minimal media supplemented with [^{15}N] ammonium chloride, [^{13}C] D-Glucose, and 7.5 mL/liter ^{13}C,^{15}N, 10x Bio-express cell growth media (Cambridge Isotope labs), A 10 μM solution of ^{15}N- labeled GACKIX was prepared in a 9:1 H_2O:D_2O, 10 mM phosphate buffer containing 100 mM NaCl at pH 7.2. ^{1}H-^{15}N HSQC experiments were recorded at 27 ^{o}C on an Avance Bruker 600 MHz NMR spectrometer equipped with a 5 mm cryogenic probe. An HSQC spectrum was collected in the absence of sekikaic acid and compared with data recorded in the presence of increasing concentration of small molecule. For increased sensitivity, ^{1}H-^{15}N HSQC spectra were folded in the nitrogen dimension. Backbone amide resonance assignments for unbound GACKIX were carried out by analysis of 3D HNCA, HNCACO, and CBCACONH NMR spectra. Data was processed using NMRpipe and analyzed in Sparky (UCSF). Reported ^{15}N chemical shifts are based on the folded resonance positions. Binding of sekikaic acid was determined from perturbation of chemical shifts observed of protein backbone amide. Chemical shift changes for individual peaks were quantified as $((0.2*\Delta\delta^{15}N)^2+ (\Delta\delta^{1}H)^2)^{0.5}$, which is a weighted length of the vector from free KIX to 5 equivalents of sekikaic acid.

Circular Dichroism

CD spectra were acquired on a J-715 spectropolarimeter equipped with a temperature control unit (Jasco, Inc., Easton, MD). Samples of 7.5 μM GACKIX were prepared in the presence and absence of sekikaic acid in 10 mM sodium phosphate pH 6.8, 100 mM NaCl, 0.1% DMSO. CD spectra were recorded at room temperature using a 1 mm pathlength quartz cuvette. Data was acquired by monitoring molar ellipticity from 200-260 nm. Reported data is the average of 5 acquired spectra. The CD signal resulting from the buffer alone was subtracted from the spectrum of each protein solution. Data were converted to mean residue ellipticity (deg cm^2 dmol^{-1}) according to the equation:

$$[\Theta] = \Psi / (1000nlc)$$

where Ψ is the CD signal in degrees, n is the number of amides, l is the path length in centimeters, and c is the concentration in decimoles per cm^3. Variable temperature CD was acquired by monitoring the molar ellipticity at 222 nm during heating of the protein

solutions at a rate of 1 degree/minute from 20-95 °C. Data analysis was performed using GraphPad Prism software.

Computational methods

Molecular dynamics simulations of the natural products in implicit solvent were performed using CHARMM.(*7*) The simulations revealed that these small molecules prefer to adopt a helical-like conformation in solution. To visually demonstrate that sekikaic acid takes on this type of conformation in solution, the small molecule conformations generated from the simulations were clustered and a representative member from each of the five largest clusters was rigidly fit using Chimera into a pseudo-density map of the p53 peptide generated using the Situs tool with an approximate resolution of 5 angstroms.(*8, 9*) As shown in Figure 5 b) in the manuscript, which was generated in Chimera, an example of a preferred conformation of sekikaic acid fits well into the pseudo-density map, which supports the idea that it behaves as a helical mimic.

Simulations were run at 300K for 1 nanosecond after an equilibration of 500 picoseconds using the Leapfrog Verlet integrator and a 1 femptosecond timestep. The CHARMM27 all-atom force field was used while the parameters for the small molecules were produced using the atom typing toolset MATCH.(*10, 11*) GBMV was used as the implicit solvent, with a constant dielectric and a 16 angstrom nonbonded cutoff. Clustering was performed using the MMTSB Tool Set with a 2 angstrom heavy atom RMSD radius.(*12*)

Cellular studies

HeLa cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% FBS. 4 x 10^4 cells/well were plated in 24-well format and allowed to adhere overnight. The next day, cells were treated with the natural products delivered in DMSO (1% final v/v). The cells were then grown for 48 h before harvesting. Western blotting was performed using a CYCLIN D1 (sc-718) or GAPDH (sc-44724) antibody. For a positive control, cells were serum-starved for 2 h prior to lysis to deplete CYCLIN D1 levels.

Table of NMR Assignments from HSQC experiments

	Free GACKIX		**GACKIX + 5eq Sekikaic Acid**	
Residue	ω_1 - ^{15}N *(ppm)*	ω_2 - ^{1}H *(ppm)*	ω_1 - ^{15}N *(ppm)*	ω_2 - ^{1}H *(ppm)*
V587	119.494	7.962	119.566	7.965
R588	125.739	8.529	125.773	8.535
W591	117.898	7.79	117.949	7.802
H592	120.638	6.859	120.685	6.863
E593	116.291	7.521	116.284	7.522
H594	114.54	7.534	114.591	7.537
V595	120.646	7.578	120.691	7.583
T596	114.848	7.438	114.822	7.441
L599	122.654	7.604	122.641	7.605
R600	118.303	7.839	118.326	7.842
S601	112.645	8.392	112.628	8.386
H602	122.482	7.951	122.484	7.951
V604	119.722	8.268	119.723	8.26
H605	117.911	7.993	117.93	7.987
L607	121.5	7.996	121.51	7.995
V608	118.594	8.208	118.509	8.184
Q609	115.924	8.128	115.924	8.128
A610	119.447	7.54	119.4	7.543
I611	114.542	7.58	114.602	7.566
F612	NA	NA	120.558	8.221
T614	114.78	7.962	114.702	7.964
D616	120.243	7.972	120.319	7.97
A618	120.534	8.28	120.539	8.281
A619	120.987	7.778	121.006	7.776
L620	117.867	7.674	117.723	7.66
K621	118.929	7.842	118.84	7.824
D622	120.197	7.898	120.197	7.898
R624	119.833	8.488	119.76	8.512
M625	118.771	8.263	118.684	8.238
E626	119.493	8.33	119.426	8.314
L628	123.188	7.911	123.254	7.92
V629	119.858	8.384	119.813	8.383
A630	120.634	7.853	120.551	7.857
Y631	120.094	7.885	120.038	7.881
A632	122.308	8.556	122.306	8.563
K633	116.008	8.682	116.007	8.674
K634	123.299	7.776	123.308	7.773

E636	120.388	8.526	120.438	8.531
D638	122.272	8.189	122.312	8.189
M639	121.051	8.05	121.01	8.046
Y640	121.466	9.273	121.55	9.281
E641	113.813	8.013	113.83	8.017
S642	112.534	7.993	112.545	7.99
A643	124.221	8.239	124.224	8.237
N644	117.259	9.17	117.268	9.176
R646	121.798	8.743	121.846	8.74
D647	117.13	8.183	117.116	8.182
Y649	121.254	7.377	121.217	7.373
Y650	115.863	8.115	115.863	8.115
H651	119.238	8.198	119.213	8.192
L652	120.361	8.992	120.387	8.99
L653	120.854	7.805	120.878	7.815
A654	121.032	8.077	121.033	8.077
E655	118.06	8.275	118.002	8.261
K656	120.43	7.901	120.366	7.905
I657	119.151	8.269	119.129	8.266
Y658	120.787	8.378	120.789	8.382
K659	118.973	8.296	118.974	8.301
I660	119.866	8.163	119.751	8.147
Q661	120.246	8.11	120.279	8.11
K662	120.024	8.149	120.013	8.179
E663	120.229	8.014	120.222	8.016
L664	119.415	8.079	119.422	8.075
E665	119.091	7.99	119.059	7.984
E666	122.324	8.025	122.274	8.023
R668	120.787	8.378	120.789	8.382
R671	118.973	8.296	118.974	8.301

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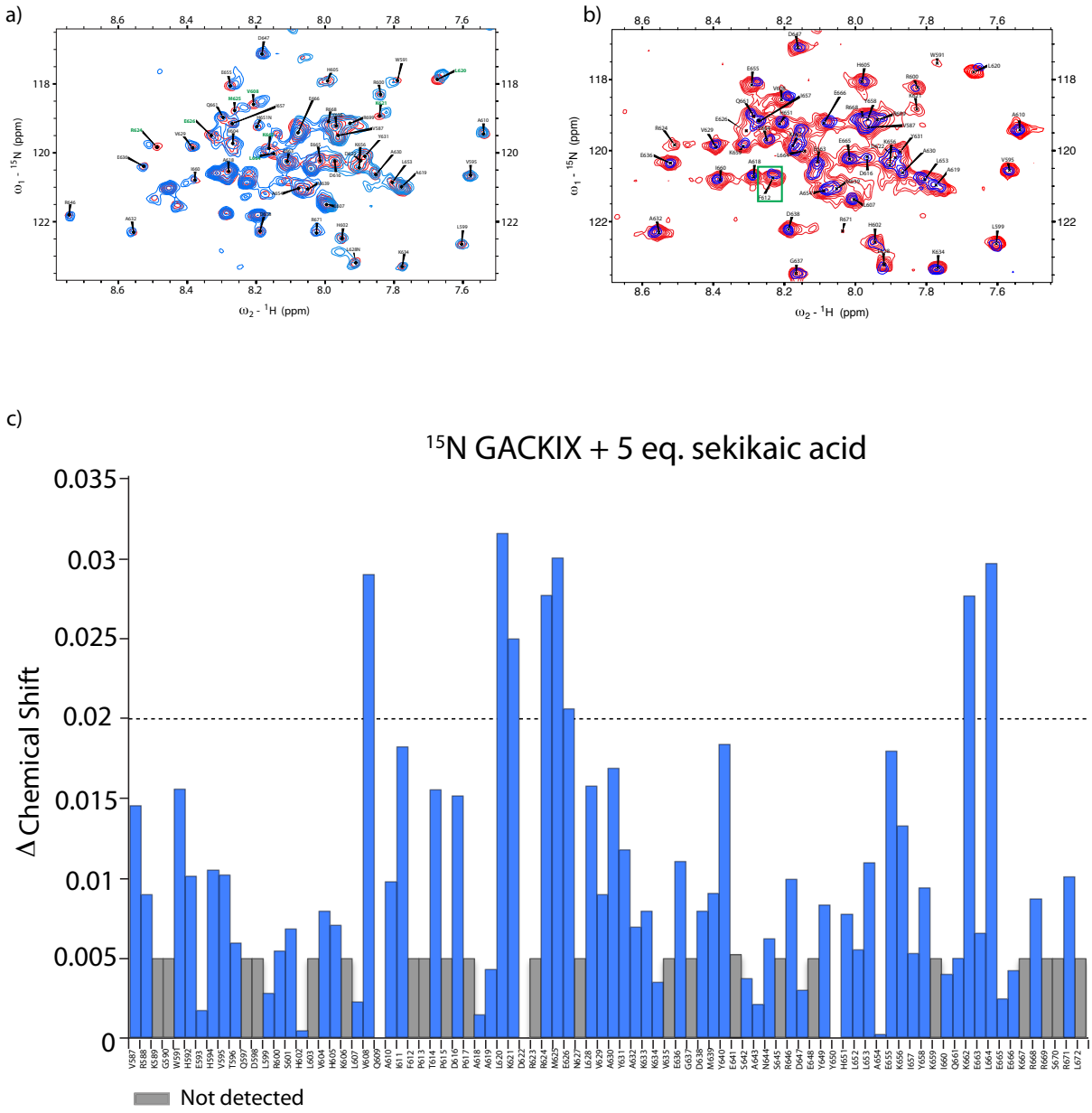
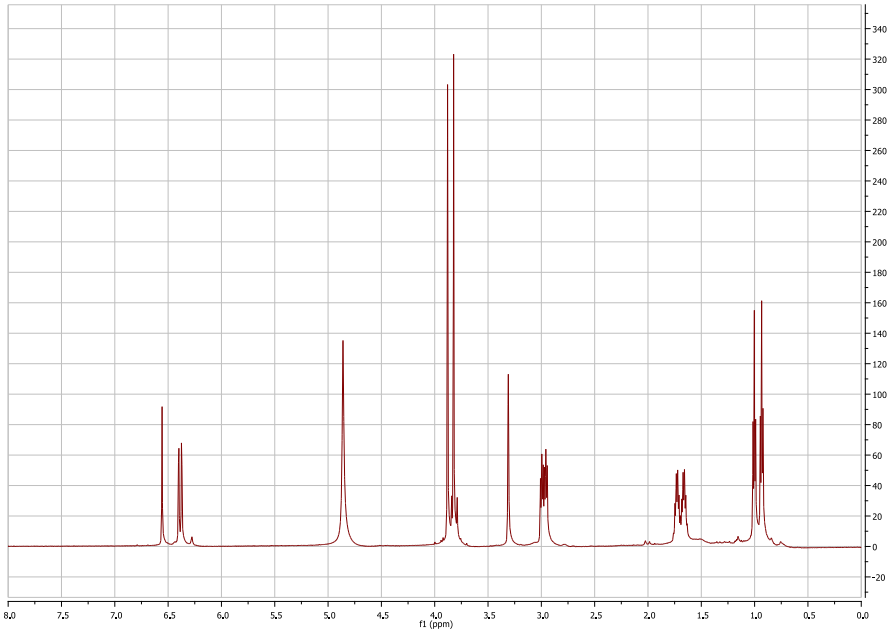


Supporting Figure 1. HSQC of sekikaic acid binding to GACKIX. a) Free ^{15}N GACKIX is shown in red and GACKIX + 5eq sekikaic acid is shown in blue. Residues that shift significantly are labeled in green. The peak arrows refer to the free GACKIX. b) An experiment similar to a) was performed with a GACKIX construct(*1*) lacking the stabilizing polar linker between the $(His)_6$ tag and GACKIX to detect the shift seen with F612 whose resonance is overlapping with a linker residue in a). In this new construct, a significant shift of 0.021 is detected for F612 when 5 eq. of sekikaic acid are added. c) Chemical shift perturbation mapping of GACKIX residues upon addition of 5 eq. of sekikaic acid seen in a). Residues that shift >0.02 are assumed to be significant, see methods for details. Residues that are unable to be detected/assigned in a) are shown in gray.

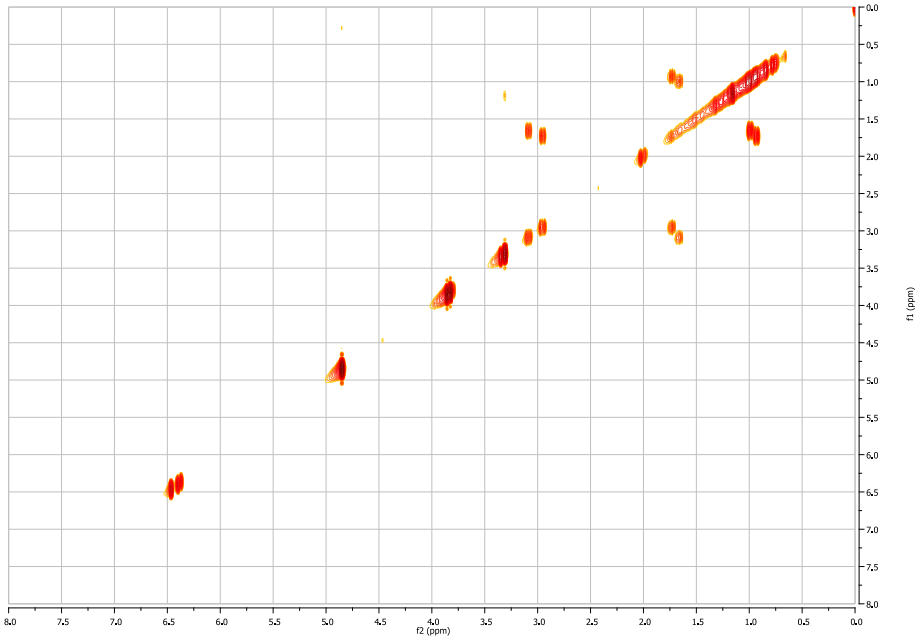
Fractionated natural product extracts		% Inhibition data in original screen		% Inhibition of MLL-KIX interaction in FP assay			% Inhibition against other FP assays				
		MLL-KIX	Counter screen	NI. = No inhibition			KIX-MLL	KIX-cMyb	Med15-VP2	Gal4-DNA	
Extract #	Type	~ 0.3 mg/mL		0.8 mg/mL	0.08 mg/mL	0.008 mg/mL	0.3 mg/mL				
1	2'-O-methylanziaic acid	88	-9	58	70	N.I.		57	20	61	
2	Fraction 1	85	15	N.I.	N.I.	N.I.					
	Fraction 2			6	N.I.	N.I.					
	Fraction 3			50	N.I.	N.I.					
	Fraction 4			65	57	N.I.	45	68	13	-20	sekikaic acid
	Fraction 5			77	8	N.I.					
	Crude			73	4	N.I.					
3	Fraction 1	78	3	N.I.	N.I.	N.I.					
	Fraction 2			31	N.I.	N.I.					
	Fraction 3			53	11	N.I.					
	Fraction 4			65	39	N.I.	38	63	29	42	
	Fraction 5			65	28	N.I.	77	72	29	27	
	Crude			74	1	N.I.					
4	Fraction 1	54	6	N.I.	N.I.	N.I.					
	Fraction 2			52	N.I.	N.I.					
	Fraction 3			37	6	N.I.					
	Fraction 4			53	84	N.I.	68	47	55	2	
	Fraction 5			54	29	N.I.	71	44	24	-17	
	Crude			62	56	2					
5	Fraction 1	46	3	N.I.	N.I.	N.I.					
	Fraction 2			67	41	N.I.					
	Fraction 3			78	25	N.I.					
	Fraction 4			67	22	N.I.					
	Fraction 5			80	64	N.I.	40	7	29	31	
	Crude			73	22	N.I.					
6	Fraction 1	65	18	N.I.	N.I.	N.I.					
	Fraction 2			71	N.I.	N.I.					
	Fraction 3			65	62	N.I.					microphyllinic acid
	Fraction 4			65	68	N.I.					
	Fraction 5			77	77	N.I.	42	73	-4	-3	4-O-demethylmicrophyllinic acid
	Crude			68	13	N.I.					
7	Fraction 1	25	8	N.I.	N.I.	N.I.					
	Fraction 2			41	N.I.	N.I.					
	Fraction 3			67	70	12	50	26	39	38	
	Fraction 4			53	36	N.I.					
	Fraction 5			49	N.I.	N.I.					
	Crude			59	N.I.	N.I.					

Supporting Figure 2. Activity of fractionated natural product extracts. Extracts active from the HTS for inhibiting fl-MLL-GACKIX were isolated from natural sources, fractionated and evaluated to confirm their activity and specificity in a series of FP-based assays: inhibition levels- high (orange), medium (yellow) and low (white). Extract 2, fraction 4 was found to contain sekikaic acid as the active component while extract 6, fraction 5 was found to contain microphyllinic acid and 4-O-demethylmicrophyllinic acid as the active components

a)

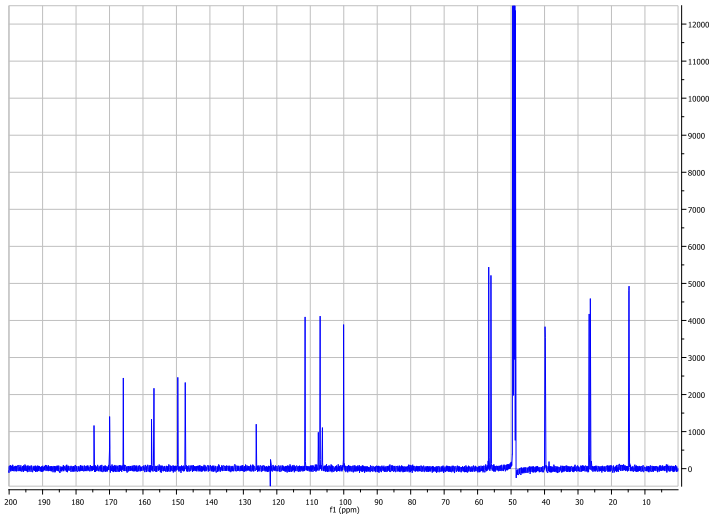


b)

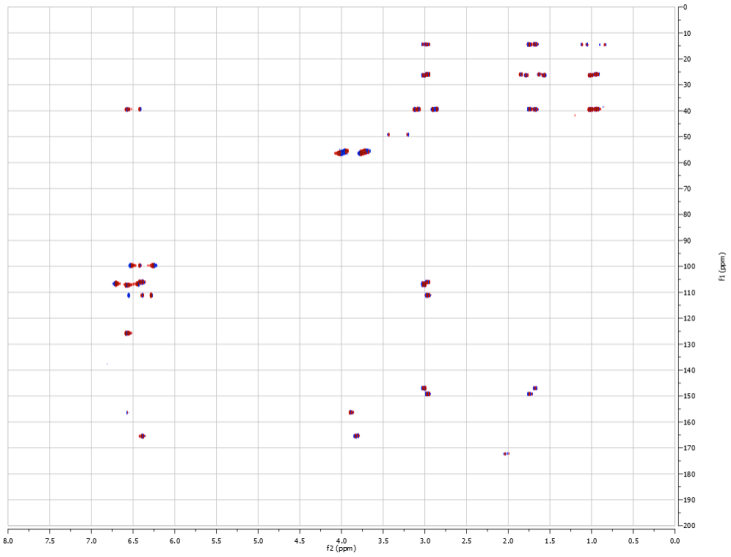


Supporting Figure 3. Characterization of extract 2, fraction 4 by NMR. a) ^{1}H NMR. Spectra recorded in CH_3OD. Reference of CH_3OD set to 3.31 ppm. b) COSY recorded in CH_3OD.

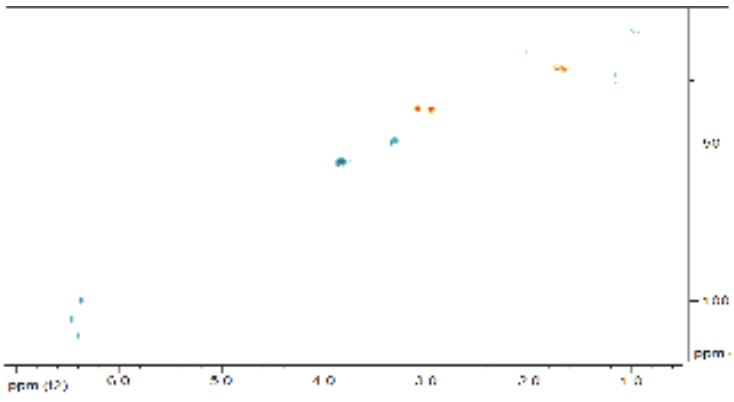
c)



d)

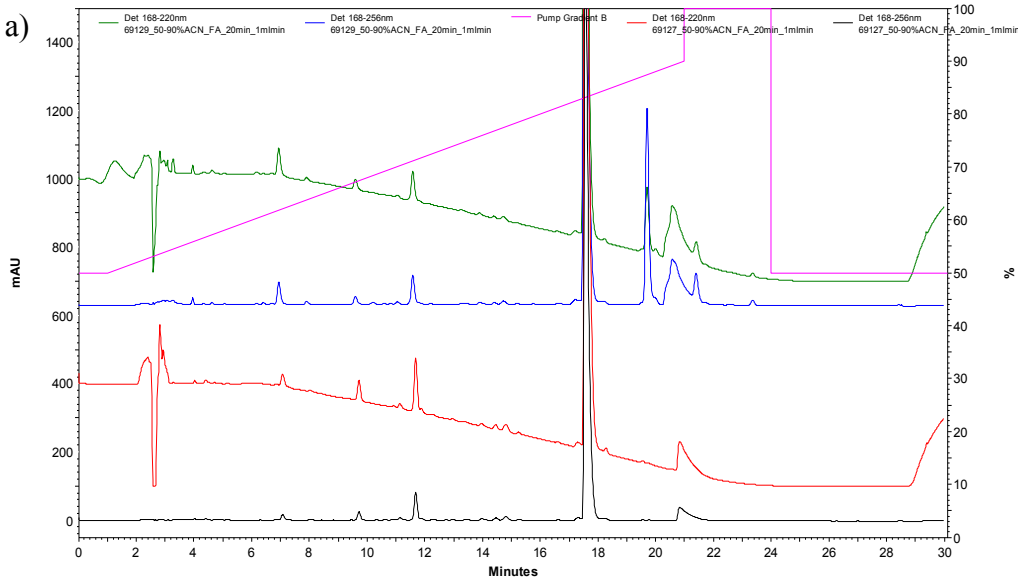


e)

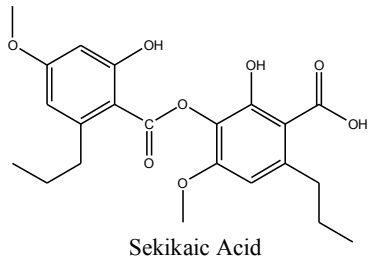


Supporting Figure 3. Characterization of extract 2, fraction 4 by NMR. c) ^{13}C NMR. Spectra recorded in CH_3OD. Reference of CH_3OD set to 49.15 ppm. d) HMBC NMR recorded in CH_3OD. e) HSQC spectra.

a)



b)

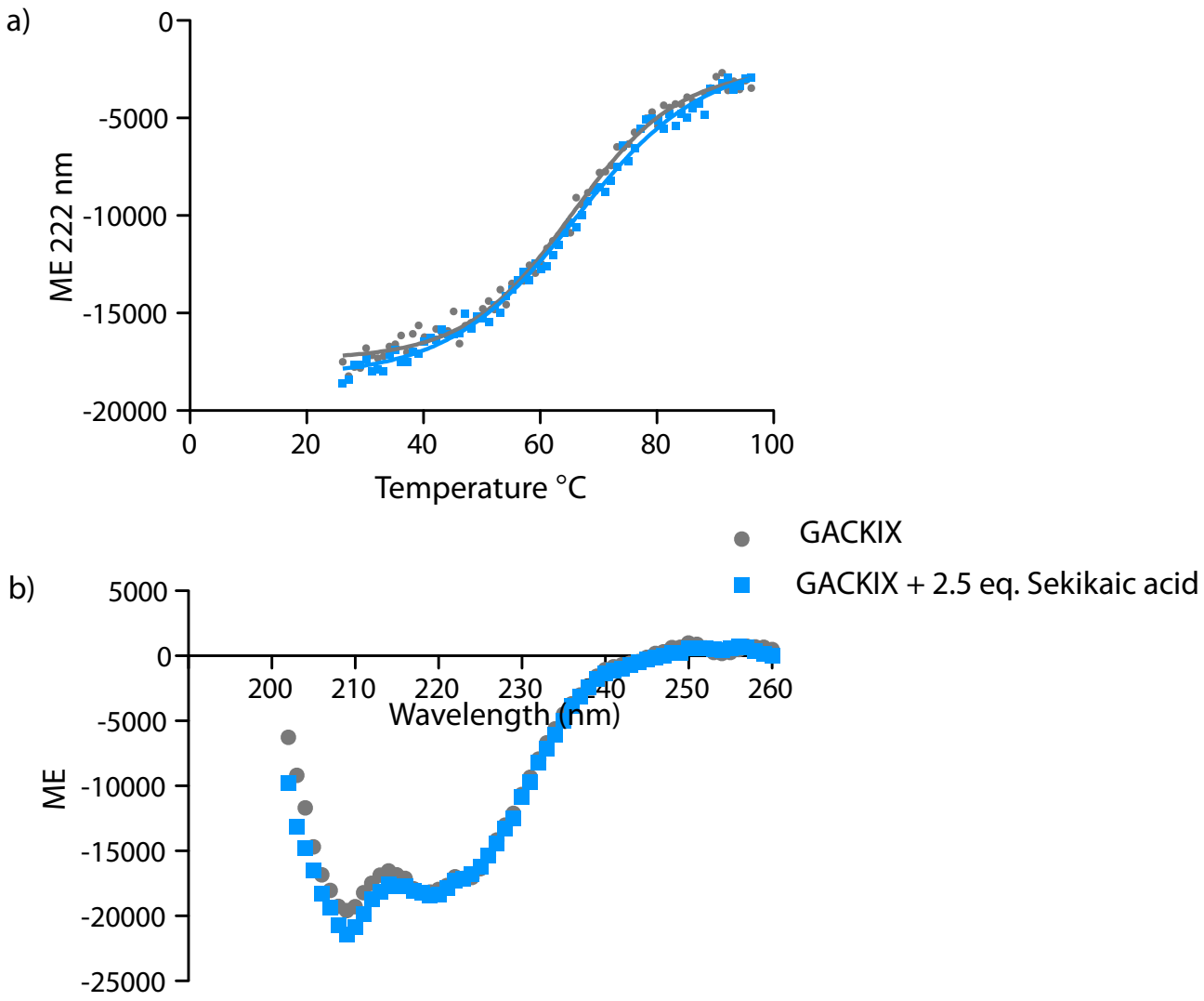


Sekikaic Acid

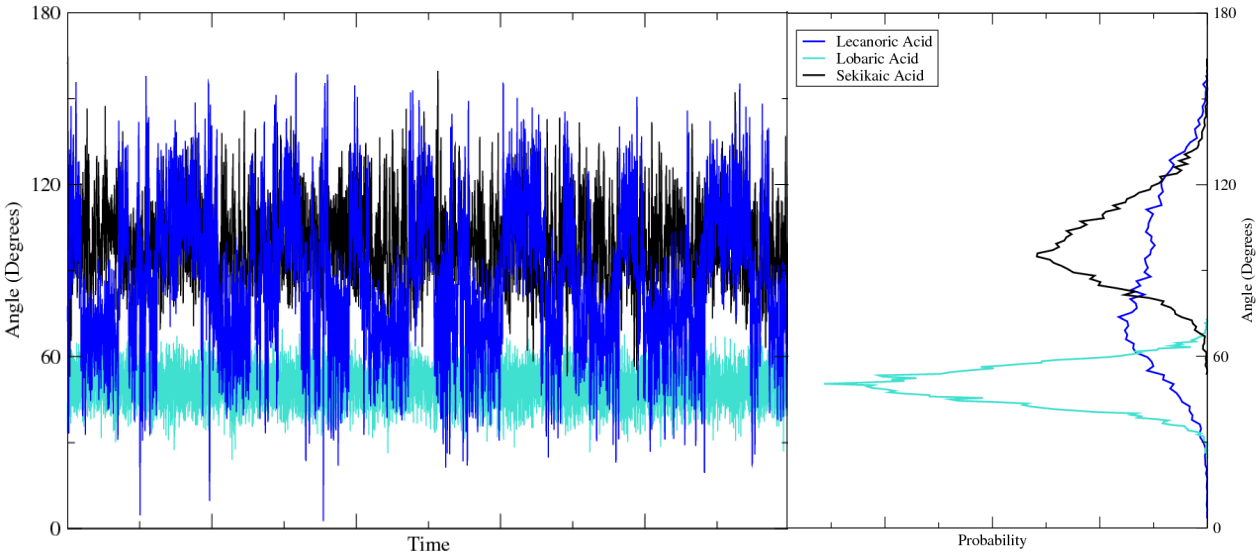
Exact mass observed $[M+Na^+]$ = 441.1517 (±1.8ppm).

Exact mass calculated for ($C_{22}H_{26}O_8Na$), $[M+Na^+]$ = 441.1525.

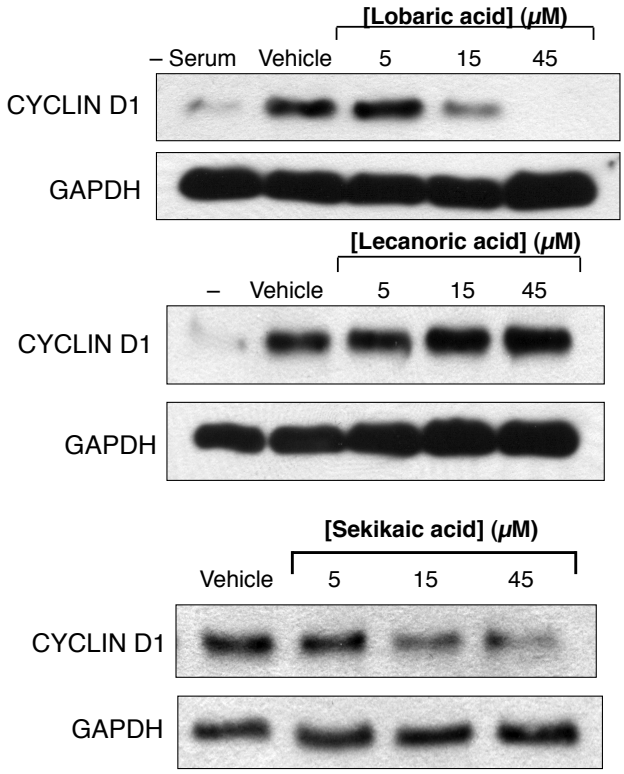
Supporting Figure 4. Characterization of extract 2, fraction 4 by HPLC and mass spectrometry. a) Reversed-phased HPLC trace using a C18 column and 50-90% acetonitrile/formic acid gradient over 20 min. Detector wavelength 220 nm (green), 256 nm (blue), 220 nm (red) and 256 nm (black). b) Structure of compound with formula $C_{22}H_{26}O_8$.



Supporting Figure 5. Impact of sekikaic acid on GACKIX secondary structure. a) Thermal melt of GACKIX in presence or absence of sekikaic acid. Circular dichroism spectra of 7.5 μM solutions of GACKIX and in 10 mM sodium phosphate pH 6.8, 100 mM NaCl at 20 ^{o}C with 22.5 μM sekikaic acid or DMSO. Protein solutions were heated from 20 to 95 ^{o}C monitoring unfolding at 222 nm. Thermal melt temperatures (T_m) were obtained using a sigmoidal function T_m 64.3 ^{o}C and 64.9 ^{o}C (with sekikaic acid). b) Full wavelength scan of GACKIX in the presence or absence of sekikaic acid.



Supporting Figure 6. Conformational rigidity of natural products. Inspection of the natural products simulations in implicit solvent showed that lecanoric acid could rotate around its ester bond more freely than sekikaic acid. To quantify this observation the angle between the two planes defined by the phenyl rings was followed throughout the trajectory (left) and the probability density of those angles sampled were plotted (right). Lecanoric acid has the largest variation in angles sampled, as seen by it's broad probability distribution which spans approximately 160 degrees. Sekikaic acid has a more narrow distribution, peaking at about 95 degrees, indicating it is not as free to rotate around its ester bond. Lobaric acid is limited to sampling angles distributed about 50 degrees as it is restrained by an additional ring.



Supporting Figure 7. Evaluation of natural products in cells. Hela cells were dosed with varying amounts of natural products for 48 h and then probed using a western blot for the c-Jun driven gene CYCLIN D1 (antibody sc-718) and the control gene GAPDH (antibody sc-44724). Sekikaic acid and lobaric acid display a dose-dependent decrease of CYCLIN D1 while GAPDH is unaffected, whereas lecanoric acid is unable to down-regulate CYCLIN D1 at the concentrations evaluated.