SUPPORTING INFORMATION

Activity-independent discovery of secondary metabolites using chemical elicitation and cheminformatic inference

Sheila M. Pimentel-Elardo[1], Dan Sørensen[2], Louis Ho[1], Mikaela Ziko[3], Stephanie A. Bueler[4], Stella Lu[5], Joe Tao[6], Arvin Moser[7], Richard Lee[7], David Agard[6], Greg Fairn[5], John L. Rubinstein[1,4], Brian K. Shoichet[8], Justin R. Nodwell[1,*]

*Corresponding author: justin.nodwell@utoronto.ca

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METHODS

Bacterial strains, culture conditions and compounds

Fifty strains were used for screening with Cl-ARC: 40 strains from the Wright Actinomycete Collection (WAC), as described by Thaker *et al.* (1); and ten sequenced strains: *Streptomyces clavuligerus* ATCC 27064, *S. ghanaensis* ATCC 14672, *S. griseoflavus* Tü 4000, *S. hygroscopicus* ATCC 53653, *S. roseosporus* NRRL 15998, *Amycolatopsis* sp. AA4, *Streptomyces* sp. Mg1, *S. sviceus* ATCC 29083, *S. viridochromogenes* DSM 40736 and *Kutzneria* sp. 744. Strains were grown on the following solid media for 7 days at 30°C: R5M (100.0 g maltose, 10.12 g $MgCl_2 \cdot H_2O$, 0.5 g K_2SO_4, 0.2 g Difco Casamino acids, 10.0 g yeast extract, 11.46 g TES, 4 ml trace elements, 10.0 ml 0.5% K_2PO_4, 4.0 ml [5M] $CaCl_2 \cdot 2H_2O$, 15.0 ml [20%] L-proline, 7.0 ml [1 M] NaOH per liter); MYM (4.0 g maltose, 4.0 g yeast extract, 10.0 g malt extract, 2.0 ml trace elements per liter), MS (20.0 g soy flour, 20.0 g mannitol, 10.0 ml [1 M] $MgCl_2 \cdot 6H_2O$ per liter), R2YE (103.0 g sucrose, 10.0 g glucose, 0.25 g K_2SO_4, 10.12 g $MgCl_2 \cdot 6H_2O$, 0.1 g Difco Casamino acids, 5.0 g yeast extract, 5.73 g TES, 2.0 ml trace elements, 10.0 ml [0.5%] K_2PO_4, 4.0 ml [5 M] $CaCl_2 \cdot 2H_2O$, 15.0 ml [20%] L-proline, 5.0 ml [1 M] NaOH per liter) and SAM (15.0 g of dextrose, 15.0 g peptone, 5.0 g NaCl, 1.0 g yeast extract, 2.5 ml glycerol per liter**).**

Comparative metabolite profiling by LC-MS analysis

Cultures growing on solid media were cut in small pieces, extracted in equal volume of *n*-butanol, sonicated for 15 min and macerated overnight. Extracts were then filtered through Whatman paper, evaporated to dryness (Genevac) and resuspended in 1:1 CH_3CN:H_2O. LC-MS analysis was performed on an Agilent 1200 series LC system coupled to a Bruker micrOTOF II with an electrospray ionization source. LC conditions were as follows: Phenomenex Kinetex C18 column (2.1 x 50 mm, 2.6 µm, 100 A°); solvents H_2O with 0.1% formic acid (A), and CH_3CN with 0.1% formic acid (B), 40°C column temperature, flow rate of 0.2 mL/min and the following gradient: 0.5 min 5% B, 5–9 min 95% B, 10–15 min 5% B. The MS conditions were set to a capillary voltage of 4.5 kV for positive mode, nebulizing gas pressure of 3 barr, dry gas flow rate (N_2) of 6 L/min and temperature at 200°C.

Isolation, purification and structure elucidation of compounds

S. ghanaensis ATCC 14672, *S. hygroscopicus* ATCC 53653 and WAC0256 were inoculated in 1 L of MYM agar, grown for 7 days at 30°C and extracted twice with *n*-butanol and evaporated to dryness. The crude extracts were subjected to pre-fractionation with Strata-X reversed phase cartridge (Phenomenex) using H_2O/CH_3CN gradient: 100% (FW), 75%:25% (F1), 50%:50% (F2), 25%:75% (F3) followed by 100% CH_3CN (F4). The resulting fractions F2, F3 and F4 which contained 9-methylstreptimidone, oxohygrolidin and the macrotetrolide series, respectively, were purified by semi-preparative HPLC (Waters XSelect CSH C18, 10 x 150 mm, 5 µm) using an Alliance 2695 HPLC series (Waters, USA). 9-methylstreptimidone eluted at 55% CH_3CN in water (0.1% formic acid) to yield ~8 mg of pure compound. Oxohygrolidin eluted at 80% CH_3CN in water (0.1% formic acid) to give ~5 mg of pure compound. The macrotetrolides eluted at 100% CH_3CN (0.1% formic acid) with yields of ~1 mg each of nonactin, monactin, dinactin and trinactin.

Chemical-genetic profiling in yeast

A library of *S. cerevisiae* haploid deletion mutants (4,309 strains) from BY4741 (*MAT*a *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*) were replicated from 384-agar arrays using a pin-replicator tool (V&P Scientific) onto YEPD agar plates (40 mg adenine, 40 mg tryptophan, 20 g peptone, 10 g yeast extract, 40 ml [50%] glucose, 1 ml [200mg/l] G-418 per liter) with either DMSO or oxohygrolidin (16 µg/ml) and incubated at 30ºC for two days. Strains that showed hypersensitivity to oxohygrolidin were further tested by serial spot dilution assay. Strains were grown overnight in YEPD and the cultures were adjusted to OD 0.1 at 600 nm and diluted 10-fold. Three microliters of the dilutions were spotted onto YEPD plates containing DMSO or 16 µg/ml oxohygrolidin and incubated at 30ºC for two days.

V-ATPase inhibition assay

V-ATPase (2 µl) was added to 160 µl total volume of 50 mM HEPES (pH 8.0), 3 mM $MgCl_2$, 0.1 % (w/v) dodecyl maltoside (Anatrace), 0.2 mM NADH, 20 units/ml pyruvate kinase, 50 units/ml L-lactic dehydrogenase, 1 mM phosphoenolpyruvate, and 2 mM ATP. The loss of NADH at 340 nm was recorded over 200 s in a SpectraMax M2 microplate reader (Molecular

Devices). 1 mM stock solutions of oxohygrolidin, bafilomycin A1 and concanamycin A were prepared in DMSO. The solvent, DMSO was added at the same concentration to the reaction mixture across all samples, with or without inhibitor. ATPase assays were repeated in triplicate and rates were determined by taking the slope of the linear region of the absorbance versus time plot for each sample.

LysoTracker Red staining assay

HeLa cells grown on coverslips were treated with oxohygrolidin in serum free DMEM for 30 min at 37°C. Cells were placed in HEPES-buffered solution HPMI with oxohygrolidin and pre-equilibrated without CO_2 for 10 min. Cells were treated with concanamycin A (Sigma) for 30 min at 37°C with a final concentration of 500 nM. Cells were stained with LysoTracker Red DND-99 at 1:5000 dilutions for 10 min at 37°C. Coverslips were washed three times and imaged immediately on an Axiovert 200M spinning disc confocal microscope (Carl Zeiss) using a 63x (NA 1.4) oil immersion objective lens. Images were captured with a back-thinned, electron-multiplied camera (C9100-13 ImagEM; Hamamatsu Photonics) using Volocity software (PerkinElmer) and LysoTracker Red was visualized using a diode-pump solid-state laser at 561 nm (Spectral Applied Sciences). Post-acquisition cells were selected using the region of interest (ROI) tool in Volocity and the total fluorescence/cell was determined. ROIs of equivalent size outside of the cell were used to subtract the background fluorescence. The majority of the fluorescence signal was due to accumulation in the lysosomes. Upon their neutralization, the signal is largely reduced.

HSP90 ATPase assay

Human Hsp90α (5 µM) was pre-incubated with the compounds at 200 µM, 100 µM or 50 µM or radicicol (50 µM) at 30 °C for ten minutes. The DMSO concentration in all reactions was normalized to 2%. After the incubation, 0.1 mM NADH (CalBiochem), 50 U/ml L-lactate dehydrogenase (CalBiochem), 1 mM phosphoenolpyruvate (Sigma) and 50 U/ml pyruvate kinase (Sigma) were added to the Hsp90α/compound mix. The reaction was initiated by adding 1 mM ATP (Sigma). The decline of NADH absorbance at 340 nm is measured in a 384-well plate (Greiner) on a microplate reader (SpectraMax M5, Molecular Devices) at 30°C.

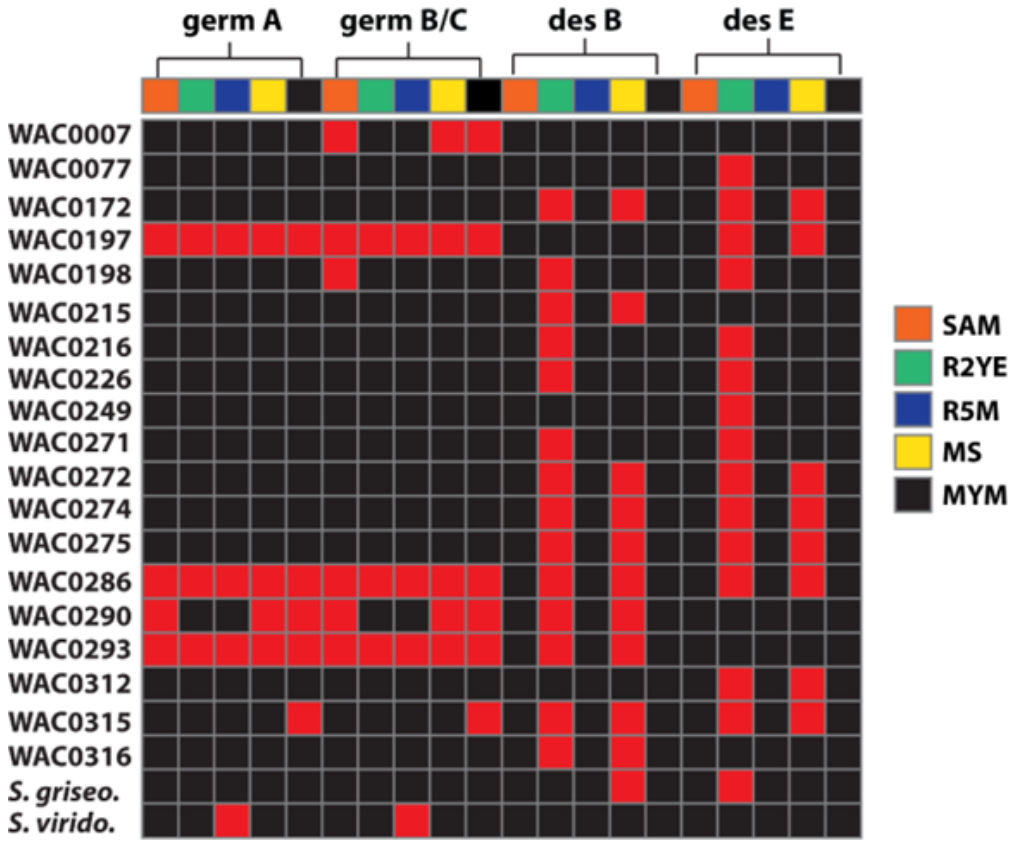


Figure S1. Germicidin A (germ A) and germicidin B/C (germ B/C) and desferrioxamine B (des B) and desferrioxamine E (des E) are commonly induced by Cl-ARC in 21 out of 50 strains.

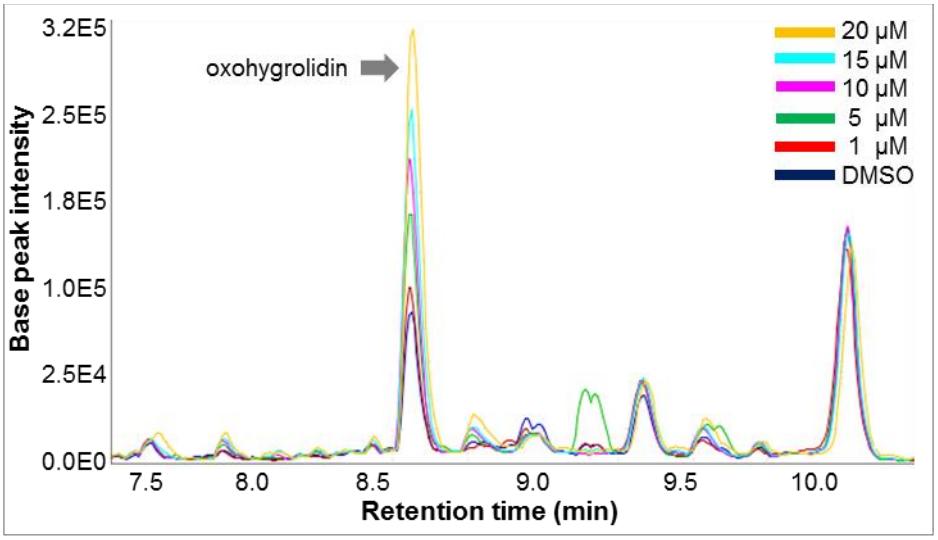


Figure S2. Dose-dependence of oxohygrolidin yields on Cl-ARC. Stepwise increases of oxohygrolidin produced by *S. ghanaensis* were observed by increasing amounts of Cl-ARC compared with DMSO.

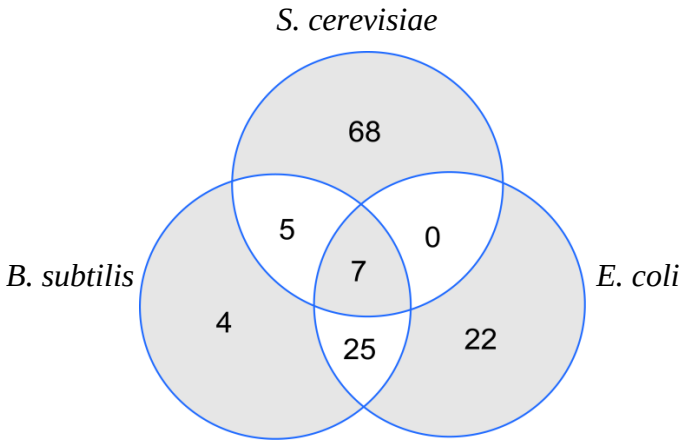
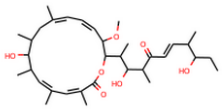


Figure S3. Crude extracts from WAC strains showing more antifungal than antibacterial activities. A total of 400 extracts were tested by disk diffusion assay against *S. cerevisiae, B. subtilis* and *E. coli.*

A

Query Molecule & ID	Target Name	Target Key	Group	Description	P-Value	MaxTC
 oxohygrolidin	VAS1_HUMAN-all	VAS1_HUMAN-all	all	VAS1_HUMAN-all	9.957e-138	0.74
	VAS1_HUMAN-other	VAS1_HUMAN-other	all	VAS1_HUMAN-other	2.017e-136	0.74
	VAS1_HUMAN-ic50	VAS1_HUMAN-ic50	all	VAS1_HUMAN-ic50	3.532e-81	0.64

B

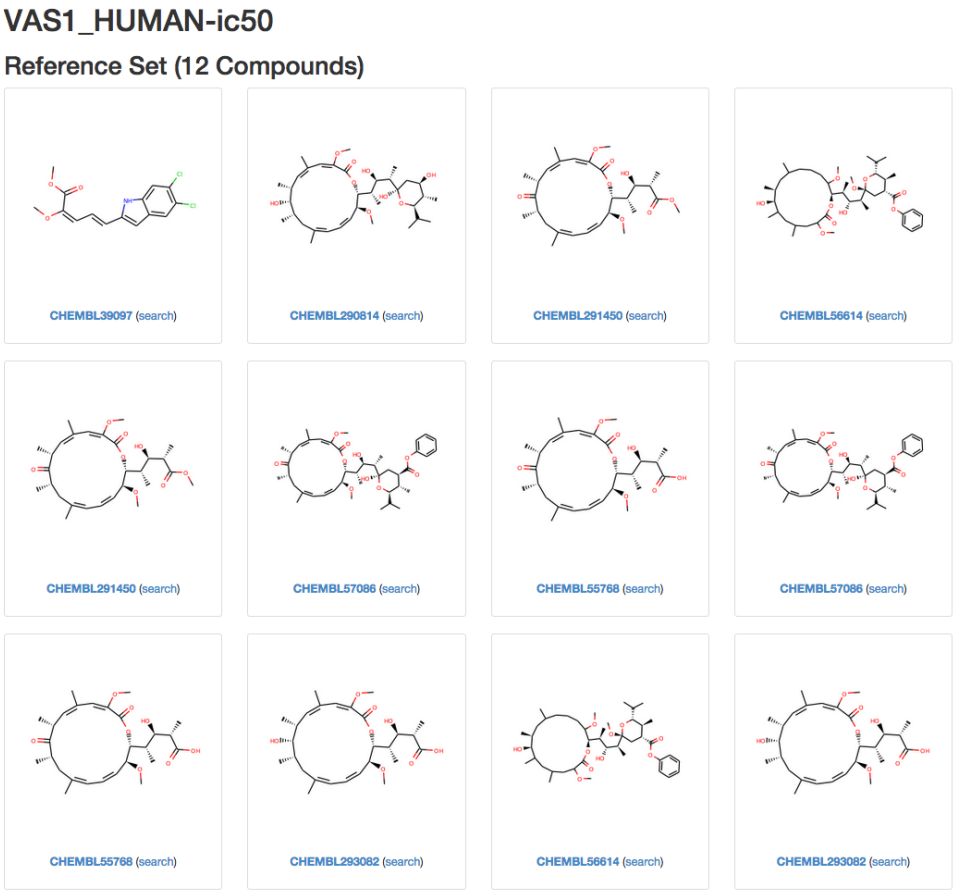


Figure S4. SEA predictions for oxohygrolidin for the vacuolar ATPase using ChEMBL20. (A) Here, P-values rather than E-values are represented. MaxTC is the Tanimoto Coefficient of the most similar V1-ATPase ligand in ChEMBL to oxohyrgolidin, using ECFP4 fingerprints. The ligands are represented as several different subsets from ChEMBL, including only those with reported IC50 values and all ligands reported in the literature. (B**)** A view of the ChEMBL V1-ATPase ligands to which oxohygrolidin was compared. Bafilomycin A1 is shown in the first row, second from left.

Table S1: List of masses (base peak ions) of compounds induced by Cl-ARC

m/z	Strain	Media	Ionization mode	Retention Time (min)	Compound
183.10	WAC007	MS	positive	7.3	germicidin B/C
183.10	WAC007	MYM	positive	7.3	germicidin B/C
183.10	WAC007	SAM	positive	7.3	germicidin B/C
197.12	WAC007	MS	positive	7.5	
197.12	WAC007	MYM	positive	7.5	germicidin A
197.12	WAC007	SAM	positive	7.5	germicidin A
601.37	WAC0077	R2YE	positive	6.5	desferrioxamine E
897.42	WAC0077	MYM	positive	8.2	
1103.44	WAC0077	MYM	negative	7.7	
427.30	WAC0165	SAM	positive	6.8	
614.33	WAC0165	SAM	positive	7.1	
796.30	WAC0165	MYM	positive	7.0	
754.49	WAC0171	MYM	positive	10.7	nonactin
754.49	WAC0171	SAM	positive	10.7	nonactin
754.49	WAC0171	R5M	positive	10.7	nonactin
754.49	WAC0171	MS	positive	10.7	nonactin
754.49	WAC0171	R2YE	positive	10.7	nonactin
768.50	WAC0171	MYM	positive	11.2	monactin
768.50	WAC0171	SAM	positive	11.2	monactin
768.50	WAC0171	R5M	positive	11.2	monactin
768.50	WAC0171	MS	positive	11.2	monactin
768.50	WAC0171	R2YE	positive	11.2	monactin
782.52	WAC0171	MYM	positive	11.7	dinactin
782.52	WAC0171	SAM	positive	11.7	dinactin
782.52	WAC0171	R5M	positive	11.7	dinactin
782.52	WAC0171	MS	positive	11.7	dinactin
782.52	WAC0171	R2YE	positive	11.7	dinactin
561.37	WAC0172	R2YE	positive	6.3	desferrioxamine B
561.37	WAC0172	MS	positive	6.3	desferrioxamine B
601.37	WAC0172	R2YE	positive	6.5	desferrioxamine E
601.37	WAC0172	MS	positive	6.5	desferrioxamine E
754.49	WAC0172	R5M	positive	10.7	monactin
768.50	WAC0172	R5M	positive	11.2	monactin
782.52	WAC0172	R5M	positive	11.7	dinactin
199.17	WAC0193	R2YE	positive	8.4	germicidin E
213.18	WAC0193	R2YE	positive	8.7	germicidin D
243.24	WAC0195	R5M	positive	10.0	

m/z	Strain	Media	Ionization mode	Retention Time (min)	Compound
243.24	WAC0195	MYM	positive	10.0	
345.30	WAC0195	MYM	positive	10.3	
183.10	WAC0197	SAM	positive	7.3	germicidin B/C
183.10	WAC0197	R2YE	positive	7.3	germicidin B/C
183.10	WAC0197	R5M	positive	7.3	germicidin B/C
183.10	WAC0197	MS	positive	7.3	germicidin B/C
183.10	WAC0197	MYM	positive	7.3	germicidin B/C
197.12	WAC0197	SAM	positive	7.5	germicidin A
197.12	WAC0197	R2YE	positive	7.5	germicidin A
197.12	WAC0197	R5M	positive	7.5	germicidin A
197.12	WAC0197	MS	positive	7.5	germicidin A
197.12	WAC0197	MYM	positive	7.5	germicidin A
229.22	WAC0197	SAM	positive	9.6	
243.24	WAC0197	SAM	positive	10.0	
243.24	WAC0197	SAM	positive	10.0	
243.24	WAC0197	R2YE	positive	10.0	
345.30	WAC0197	SAM	positive	10.3	
571.29	WAC0197	SAM	negative	8.8	
585.31	WAC0197	SAM	negative	9.3	
601.37	WAC0197	R2YE	positive	6.5	desferrioxamine E
601.37	WAC0197	MS	positive	6.5	desferrioxamine E
183.10	WAC0198	SAM	positive	7.3	germicidin B/C
197.12	WAC0198	SAM	positive	7.5	germicidin A
561.37	WAC0198	R2YE	positive	6.3	desferrioxamine B
601.37	WAC0198	R2YE	positive	6.5	desferrioxamine E
254.13	WAC0210	SAM	positive	8.3	
268.10	WAC0210	SAM	negative	7.8	
561.37	WAC0215	R2YE	positive	6.3	desferrioxamine B
561.37	WAC0215	MS	positive	6.3	desferrioxamine B
363.19	WAC0216	R2YE	positive	7.7	
380.19	WAC0216	R2YE	positive	7.5	
561.37	WAC0216	R2YE	positive	6.3	desferrioxamine B
601.37	WAC0216	R2YE	positive	6.5	desferrioxamine E
243.24	WAC0226	MYM	positive	10.0	
271.27	WAC0226	MYM	positive	10.0	
303.24	WAC0226	MS	positive	10.0	linoleic acid
317.27	WAC0226	MYM	positive	9.3	
345.30	WAC0226	MYM	positive	10.3	
561.37	WAC0226	R2YE	positive	6.3	desferrioxamine B

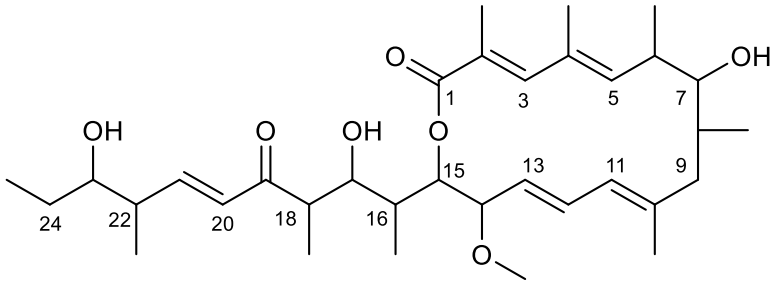
m/z	Strain	Media	Ionization mode	Retention Time (min)	Compound
601.37	WAC0226	R2YE	positive	6.5	desferrioxamine E
866.47	WAC0226	MS	positive	8.6	
897.42	WAC0226	MYM	positive	8.2	
1072.63	WAC0226	MS	positive	11.7	
923.56	WAC0236	MYM	negative	11.3	
937.58	WAC0236	MYM	negative	11.9	
934.54	WAC0243	R2YE	positive	8.5	
948.55	WAC0243	R2YE	positive	8.7	
601.37	WAC0249	R2YE	positive	6.5	desferrioxamine E
897.42	WAC0249	SAM	positive	8.2	
897.42	WAC0249	MYM	positive	8.2	
754.49	WAC0256	MYM	positive	10.7	nonactin
754.49	WAC0256	SAM	positive	10.7	nonactin
754.49	WAC0256	R5M	positive	10.7	nonactin
754.49	WAC0256	MS	positive	10.7	nonactin
754.49	WAC0256	R2YE	positive	10.7	nonactin
768.50	WAC0256	MYM	positive	11.2	monactin
768.50	WAC0256	SAM	positive	11.2	monactin
768.50	WAC0256	R5M	positive	11.2	monactin
768.50	WAC0256	MS	positive	11.2	monactin
768.50	WAC0256	R2YE	positive	11.2	monactin
782.52	WAC0256	MYM	positive	11.7	dinactin
782.52	WAC0256	SAM	positive	11.7	dinactin
782.52	WAC0256	R5M	positive	11.7	dinactin
782.52	WAC0256	MS	positive	11.7	dinactin
782.52	WAC0256	R2YE	positive	11.7	dinactin
897.42	WAC0256	MYM	positive	8.2	
754.49	WAC0269	MYM	positive	10.7	nonactin
768.50	WAC0269	MYM	positive	11.2	monactin
782.52	WAC0269	MYM	positive	11.7	dinactin
561.37	WAC0271	R2YE	positive	6.3	desferrioxamine B
601.37	WAC0271	R2YE	positive	6.5	desferrioxamine E
243.24	WAC0272	MYM	positive	10.0	
561.37	WAC0272	R2YE	positive	6.3	desferrioxamine B
561.37	WAC0272	MS	positive	6.3	desferrioxamine B
601.37	WAC0272	R2YE	positive	6.5	desferrioxamine E
601.37	WAC0272	MS	positive	6.5	desferrioxamine E
897.42	WAC0272	R2YE	positive	8.2	
561.37	WAC0274	R2YE	positive	6.3	desferrioxamine B

m/z	Strain	Media	Ionization mode	Retention Time (min)	Compound
561.37	WAC0274	MS	positive	6.3	desferrioxamine B
601.37	WAC0274	MS	positive	6.5	desferrioxamine E
601.37	WAC0274	R2YE	positive	6.5	desferrioxamine E
561.37	WAC0275	R2YE	positive	6.3	desferrioxamine B
561.37	WAC0275	MS	positive	6.3	desferrioxamine B
601.37	WAC0275	MS	positive	6.5	desferrioxamine E
601.37	WAC0275	R2YE	positive	6.5	desferrioxamine E
183.10	WAC0286	SAM	positive	7.3	germicidin B/C
183.10	WAC0286	R2YE	positive	7.3	germicidin B/C
183.10	WAC0286	R5M	positive	7.3	germicidin B/C
183.10	WAC0286	MS	positive	7.3	germicidin B/C
183.10	WAC0286	MYM	positive	7.3	germicidin B/C
197.12	WAC0286	SAM	positive	7.5	germicidin A
197.12	WAC0286	R2YE	positive	7.5	germicidin A
197.12	WAC0286	R5M	positive	7.5	germicidin A
197.12	WAC0286	MS	positive	7.5	germicidin A
197.12	WAC0286	MYM	positive	7.5	germicidin A
561.37	WAC0286	R2YE	positive	6.3	desferrioxamine B
561.37	WAC0286	MS	positive	6.3	desferrioxamine B
601.37	WAC0286	R2YE	positive	6.5	desferrioxamine E
601.37	WAC0286	MS	positive	6.5	desferrioxamine E
1058.57	WAC0286	MS	positive	8.3	
243.24	WAC0287	MYM	positive	10.0	
247.12	WAC0287	MYM	positive	9.9	
183.10	WAC0290	MYM	positive	7.3	germicidin B/C
183.10	WAC0290	MS	positive	7.3	germicidin B/C
183.10	WAC0290	SAM	positive	7.3	germicidin B/C
197.12	WAC0290	MYM	positive	7.5	germicidin A
197.12	WAC0290	MS	positive	7.5	germicidin A
197.12	WAC0290	SAM	positive	7.5	germicidin A
199.17	WAC0290	MYM	positive	8.4	germicidin E
199.17	WAC0290	MS	positive	8.4	germicidin E
213.18	WAC0290	MYM	positive	8.7	germicidin D
213.18	WAC0290	MS	positive	8.7	germicidin D
561.37	WAC0290	MS	positive	6.3	desferrioxamine B
561.37	WAC0290	R2YE	positive	6.3	desferrioxamine B
897.42	WAC0290	MYM	positive	8.2	
183.10	WAC0293	SAM	positive	7.3	germicidin B/C
183.10	WAC0293	R5M	positive	7.3	germicidin B/C

m/z	Strain	Media	Ionization mode	Retention Time (min)	Compound
183.10	WAC0293	MS	positive	7.3	germicidin B/C
183.10	WAC0293	MYM	positive	7.3	germicidin B/C
197.12	WAC0293	SAM	positive	7.5	germicidin A
197.12	WAC0293	R5M	positive	7.5	germicidin A
197.12	WAC0293	MS	positive	7.5	germicidin A
197.12	WAC0293	MYM	positive	7.5	germicidin A
199.17	WAC0293	SAM	positive	8.4	germicidin E
213.18	WAC0293	SAM	positive	8.7	germicidin D
243.24	WAC0293	R5M	positive	10.0	
317.27	WAC0293	R5M	positive	9.3	
343.27	WAC0293	R5M	positive	10.0	
353.29	WAC0293	R5M	positive	10.0	
561.37	WAC0293	R2YE	positive	6.3	desferrioxamine B
561.37	WAC0293	MS	positive	6.3	desferrioxamine B
897.42	WAC0293	R5M	positive	8.2	
427.30	WAC0295	SAM	positive	6.8	
427.30	WAC0295	R2YE	positive	6.8	
427.30	WAC0295	R5M	positive	6.8	
614.33	WAC0295	SAM	positive	7.1	
614.33	WAC0295	R2YE	positive	7.1	
614.33	WAC0295	R5M	positive	7.1	
253.06	WAC0312	SAM	negative	7.1	
313.17	WAC0312	SAM	negative	7.6	
468.21	WAC0312	SAM	negative	7.7	
504.24	WAC0312	SAM	negative	7.4	
601.37	WAC0312	R2YE	positive	6.5	desferrioxamine E
601.37	WAC0312	MS	positive	6.5	desferrioxamine E
183.10	WAC0315	MYM	positive	7.3	germicidin B/C
197.12	WAC0315	MYM	positive	7.5	germicidin A
199.17	WAC0315	MYM	positive	8.4	germicidin E
213.18	WAC0315	MYM	positive	8.7	germicidin D
419.24	WAC0315	MYM	positive	9.9	
561.37	WAC0315	R2YE	positive	6.3	desferrioxamine B
561.37	WAC0315	MS	positive	6.3	desferrioxamine B
601.37	WAC0315	R2YE	positive	6.5	desferrioxamine E
601.37	WAC0315	MS	positive	6.5	desferrioxamine E
199.17	WAC0316	R2YE	positive	8.4	germicidin E
199.17	WAC0316	MS	positive	8.4	germicidin E
213.18	WAC0316	R2YE	positive	8.7	germicidin D

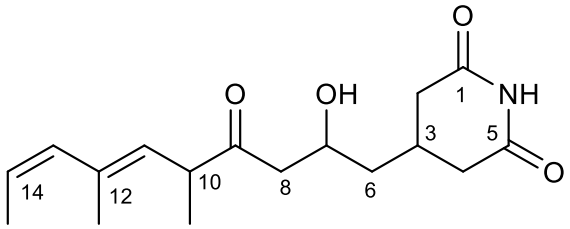
m/z	Strain	Media	Ionization mode	Retention Time (min)	Compound
213.18	WAC0316	MS	positive	8.7	germicidin D
427.30	WAC0316	SAM	positive	6.8	
427.30	WAC0316	R2YE	positive	6.8	
561.37	WAC0316	R2YE	positive	6.3	desferrioxamine B
561.37	WAC0316	MS	positive	6.3	desferrioxamine B
614.33	WAC0316	SAM	positive	7.1	
614.33	WAC0316	R2YE	positive	7.1	
427.30	WAC04659	R5M	positive	6.8	
614.33	WAC04659	R5M	positive	7.1	
923.56	WAC04741	R2YE	negative	11.3	
937.58	WAC04741	R2YE	negative	11.9	
373.24	*S. ghanaensis*	MYM	positive	9.3	
543.37	*S. ghanaensis*	MYM	positive	8.6	oxohygrolidin
561.37	*S. griseoflavus*	MS	positive	6.3	desferrioxamine B
306.17	*S. hygroscopicus*	MYM	positive	7.0	
308.17	*S. hygroscopicus*	MYM	positive	7.3	9-methylstreptimidone
243.24	*S. roseosporus*	MYM	positive	10.0	
839.46	*S. roseosporus*	MYM	positive	8.0	arylomycin
335.22	*S.* sp. Mg1	R5M	positive	8.1	
923.56	*S.* sp. Mg1	MYM	negative	11.3	
937.58	*S.* sp. Mg1	MYM	negative	11.9	
183.10	*S. viridochromogenes*	R5M	positive	7.3	germicidin B/C
197.12	*S. viridochromogenes*	R5M	positive	7.5	germicidin A

Table S2: ^{13}C (176.08 MHz) and ^{1}H NMR (700.17 MHz) data of oxohygrolidin in DMSO-d_6



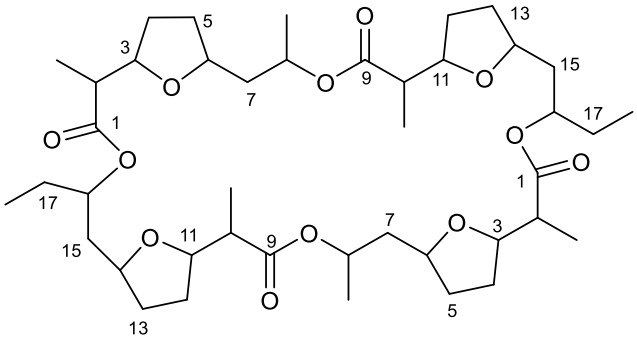
Position	δ_C	δ_H	mult (J_{H-H})
1	168.9		
2	121.0		
2-Me	13.1	1.96	s
3	145.3	7.13	s
4	132.1		
4-Me	14.5	1.89	s
5	145.7	5.93	d (8.6)
6	36.8	2.44	m
6-Me	17.8	0.96	d (7.0)
7	77.7	3.18	s
8	38.7	1.72	m
8-Me	22.4	0.88	d (7.0)
9	41.0	2.08	m
		1.86	m
10	141.7		
10-Me	18.5	1.73	s
11	123.3	5.72	d (11.0)
12	131.0	6.46	dd (15.0, 11.0)
13	124.7	5.16	dd (15.0, 7.8)
14	82.7	3.96	m
14-OMe	55.1	3.13	s
15	74.1	5.10	dd (5.8, 2.8)
16	39.3	1.82	m
16-Me	11.1	0.86	d (7.0)
17	70.5	3.65	m
18	46.0	2.91	m
18-Me	9.2	0.97	d (5.4)
19	201.5		
20	127.2	6.17	dd (15.9, 0.9)
21	149.0	6.70	dd (15.9, 8.1)
22	42.1	2.29	m
22-Me	15.1	0.97	d (5.4)
23	73.9	3.24	m
24	26.7	1.19	m
		1.36	m
24-Me	10.0	0.83	t (7.4)

Table S3: ^{13}C (176.08 MHz) and ^{1}H NMR (700.17 MHz) data of 9-methylstreptimidone in DMSO-d_6



Position	δ_C	δ_H	mult (J_{H-H})
1	173.3		
2	36.9	2.26	m
		2.24	m
3	26.6	2.19	m
4	41.6	1.29	m
		1.35	m
5	173.2		
6	38.0	2.17	m
		2.22	m
7	63.6	3.95	m
8	48.7	2.47	m
		2.55	m
9	209.6		
10	45.7	3.52	dq (9.6, 6.8)
10-Me	16.1	1.05	d (6.8)
11	129.3	5.16	dm (9.6)
12	134.2		
12-Me	16.9	1.80	dd (1.2, 0.7)
13	133.1	5.82	dq (11.8)
14	124.3	5.44	dq (11.8, 7.2)
14-Me	14.6	1.74	dd (7.2, 1.8)

Table S4: ^{13}C (176.08 MHz) and ^{1}H NMR (700.17 MHz) data of dinactin in DMSO-d_6



Position	δ_C	δ_H	mult (J_{H-H})
1	173.9		
2	45.1	2.43	m
3	80.2	3.89	m
4	28.1	1.57	m
5	31.3	1.95	m
6	76.0	3.77	m
7	42.27	1.72	m
		1.64	m
8	68.9	4.82	m
8-Me	20.6	1.16	d (6.4)
9	173.52		
10	45.2	2.44	m
10-Me	13.3	0.99	d (6.9)
11	80.2	3.89	m
12	28.2	1.87	m
13	31.4	1.42	m
14	76.0	3.77	m
15	40.1	1.65	td (8.0, 8.0, 2.9)
16	72.9	4.78	m
17	27.3	1.59	m
		1.48	m
18	9.6	0.82	m

Table S5. List of genes and their functions in *S. cerevisiae**

Gene	Function
VRP1	Proline-rich actin-associated protein involved in cytoskeletal organization and cytokinesis; related to mammalian Wiskott-Aldrich syndrome protein
VPS1	Dynamin-like GTPase required for vacuolar sorting; also involved in actin cytoskeleton organization, endocytosis, late Golgi-retention of some proteins, regulation of peroxisome biogenesis
SMI1	Protein involved in the regulation of cell wall synthesis; proposed to be involved in coordinating cell cycle progression with cell wall integrity
UBP3	Ubiquitin-specific protease involved in transport and osmotic response; interacts with Bre5p to co-regulate anterograde and retrograde transport
GCS1	ADP-ribosylation factor GTPase activating protein (ARF GAP), involved in ER-Golgi transport; shares functional similarity with Glo3p
DRS2	Aminophospholipid translocase (flippase) that maintains membrane lipid asymmetry in post-Golgi secretory vesicles; contributes to clathrin-coated vesicle formation and endocytosis; mutations in human homolog ATP8B1 result in liver disease
VPS52	Component of the GARP (Golgi-associated retrograde protein) complex, Vps51p-Vps52p-Vps53p-Vps54p, which is required for the recycling of proteins from endosomes to the late Golgi; involved in localization of actin and chitin
THI22	Protein with similarity to hydroxymethylpyrimidine phosphate kinases; member of a gene family with THI20 and THI21; not required for thiamine biosynthesis
KEX2	Subtilisin-like protease (proprotein convertase), a calcium-dependent serine protease involved in the activation of proproteins of the secretory pathway
MSC1	Protein of unknown function; mutant is defective in directing meiotic recombination events to homologous chromatids; the authentic, non-tagged protein is detected in highly purified mitochondria and is phosphorylated
VPS9	A guanine nucleotide exchange factor involved in vesicle-mediated vacuolar protein transport; specifically stimulates the intrinsic guanine nucleotide exchange activity of Vps21p/Rab5: similar to mammalian *Ras* inhibitors; binds ubiquitin
FPR3	Nucleolar peptidyl-prolyl cis-trans isomerase (PPIase); FK506 binding protein; phosphorylated by casein kinase II (Cka1p-Cka2p-Ckb1p-Ckb2p) and dephosphorylated by Ptp1p
MDM30	F-box component of an SCF ubiquitin protein ligase complex; associates with and is required for Fzo1p ubiquitination and for mitochondria fusion; stimulates nuclear export of specific mRNAs; promotes ubiquitin-mediated degradation of Gal4p in some strains

*as annotated in the *Saccharomyces* Genome Database (www.yeastgenome.org)