

Supplementary Material-Statistical Analysis

Tabla 1. Percentage of Ergosterol present in *Ustilago maydis* FB₁ cultures treated with HMGR inhibitors

μM	Simvastatin			Control W/I			Control DMSO			α--asarone			Compound 1			Compound 2					
	% Erg	LCL95 %	UCL95 %	% Erg	LCL95 %	UCL95 %	% Erg	LCL95 %	UCL95 %	% Erg	LCL95 %	UCL95 %	% Erg	LCL95 %	UCL95 %	% Erg	LCL95 %	UCL95 %			
10	99±1.0	97	102	76±1.3	73	79	61±0.6	59	62	83±1.8	78	87	76±1.3	73	79	61±0.6	59	62	83±1.8	78	87
50	97±2.7	90	104	65±0.4	64	66	52±0.9	50	54	76±1.1	73	78	65±0.4	64	66	52±0.9	50	54	76±1.1	73	78
100	80±0.8	78	82	48±0.7	46	49	50±1.4	47	54	65±1.2	62	68	48±0.7	46	49	50±1.4	47	54	65±1.2	62	68
200	67±2.1	63	73	37±1.0	35	40	43±0.7	41	44	42±1.2	39	44	37±1.0	35	40	43±0.7	41	44	42±1.2	39	44
300	24±0.8	22	26	25±1.2	22	28	36±1.4	32	39	34±1.9	31	36	25±1.2	22	28	36±1.4	32	39	34±1.9	31	36

For all the measured parameters, averages and standard deviations were obtained. The differences between the means were analyzed with a Student's *t* test to obtain P values that represented statistically significant differences. The experimental data were considered significant with $P < 0.05$ (*), being very significant with $P < 0.01$ (**) and extremely significant with $P < 0.001$ (***). The confidence intervals of 95% indicate that within the given range is the real value of a parameter with 95% certainty. The comparison of multiple groups was performed using the two-way ANOVA analysis of variance, followed by the Tukey test. The analysis and graph were made with the GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA). Upper control limit (UCL) and lower control limit (LCL) were calculated to verify whether the values of the samples are within the control limits.

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	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
W/I vs. DMSO	No	>0.9999	DMSO vs. W/I	No	>0.9999
W/I vs. 10: Simvastatin	No	>0.9999	DMSO vs. 10: Simvastatin	No	>0.9999
W/I vs. 10: α -asarone	Yes	<0.0001	DMSO vs. 10: α -asarone	Yes	<0.0001
W/I vs. 10:1	Yes	<0.0001	DMSO vs. 10:1	Yes	<0.0001
W/I vs. 10:2	Yes	<0.0001	DMSO vs. 10:2	Yes	<0.0001
W/I vs. 50: Simvastatin	No	0.1159	DMSO vs. 50: Simvastatin	No	0.1159
W/I vs. 50: α -asarone	Yes	<0.0001	DMSO vs. 50: α -asarone	Yes	<0.0001
W/I vs. 50:1	Yes	<0.0001	DMSO vs. 50:1	Yes	<0.0001
W/I vs. 50:2	Yes	<0.0001	DMSO vs. 50:2	Yes	<0.0001
W/I vs. 100: Simvastatin	Yes	<0.0001	DMSO vs. 100: Simvastatin	Yes	<0.0001
W/I vs. 100: α -asarone	Yes	<0.0001	DMSO vs. 100: α -asarone	Yes	<0.0001
W/I vs. 100:1	Yes	<0.0001	DMSO vs. 100:1	Yes	<0.0001
W/I vs. 100:2	Yes	<0.0001	DMSO vs. 100:2	Yes	<0.0001
W/I vs. 200: Simvastatin	Yes	<0.0001	DMSO vs. 200: Simvastatin	Yes	<0.0001
W/I vs. 200: α -asarone	Yes	<0.0001	DMSO vs. 200: α -asarone	Yes	<0.0001
W/I vs. 200:1	Yes	<0.0001	DMSO vs. 200:1	Yes	<0.0001
W/I vs. 200:2	Yes	<0.0001	DMSO vs. 200:2	Yes	<0.0001
W/I vs. 300: Simvastatin	Yes	<0.0001	DMSO vs. 300: Simvastatin	Yes	<0.0001
W/I vs. 300: α -asarone	Yes	<0.0001	DMSO vs. 300: α -asarone	Yes	<0.0001
W/I vs. 300:1	Yes	<0.0001	DMSO vs. 300:1	Yes	<0.0001
W/I vs. 300:2	Yes	<0.0001	DMSO vs. 300:2	Yes	<0.0001

	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
10:Simvastatin vs. W/I	No	>0.9999	50:Simvastatin vs. W/I	No	0.1159
10:Simvastatin vs. DMSO	No	>0.9999	50:Simvastatin vs. DMSO	No	0.1159
10:Simvastatin vs. 10: α -asarone	Yes	<0.0001	50:Simvastatin vs. 50: α -asarone	Yes	<0.0001
10:Simvastatin vs. 10:1	Yes	<0.0001	50:Simvastatin vs. 50:1	Yes	<0.0001
10:Simvastatin vs. 10:2	Yes	<0.0001	50:Simvastatin vs. 50:2	Yes	<0.0001
10:Simvastatin vs. 50:Simvastatin	No	0.8191	50:Simvastatin vs. 100:Simvastatin	Yes	<0.0001
10:Simvastatin vs. 50: α -asarone	Yes	<0.0001	50:Simvastatin vs. 100: α -asarone	Yes	<0.0001
10:Simvastatin vs. 50:1	Yes	<0.0001	50:Simvastatin vs. 100:1	Yes	<0.0001
10:Simvastatin vs. 50:2	Yes	<0.0001	50:Simvastatin vs. 100:2	Yes	<0.0001
10:Simvastatin vs. 100:Simvastatin	Yes	<0.0001	50:Simvastatin vs. 200:Simvastatin	Yes	<0.0001
10:Simvastatin vs. 100: α -asarone	Yes	<0.0001	50:Simvastatin vs. 200: α -asarone	Yes	<0.0001
10:Simvastatin vs. 100:1	Yes	<0.0001	50:Simvastatin vs. 200:1	Yes	<0.0001
10:Simvastatin vs. 100:2	Yes	<0.0001	50:Simvastatin vs. 200:2	Yes	<0.0001
10:Simvastatin vs. 200:Simvastatin	Yes	<0.0001	50:Simvastatin vs. 300:Simvastatin	Yes	<0.0001
10:Simvastatin vs. 200: α -asarone	Yes	<0.0001	50:Simvastatin vs. 300: α -asarone	Yes	<0.0001
10:Simvastatin vs. 200:1	Yes	<0.0001	50:Simvastatin vs. 300:1	Yes	<0.0001
10:Simvastatin vs. 200:2	Yes	<0.0001	50:Simvastatin vs. 300:2	Yes	<0.0001
10:Simvastatin vs. 300:Simvastatin	Yes	<0.0001			
10:Simvastatin vs. 300: α -asarone	Yes	<0.0001			
10:Simvastatin vs. 300:1	Yes	<0.0001			
10:Simvastatin vs. 300:2	Yes	<0.0001			

	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
100:Simvastatin vs. 200:W/I	Yes	<0.0001	200:Simvastatin vs. DMSO	Yes	<0.0001
100:Simvastatin vs. 200:DMSO	Yes	<0.0001	200:Simvastatin vs. 300:Simvastatin	Yes	<0.0001
100:Simvastatin vs. 100: α -asarone	Yes	<0.0001	200:Simvastatin vs. 200: α -asarone	Yes	<0.0001
100:Simvastatin vs. 100:1	Yes	<0.0001	200:Simvastatin vs. 200:1	Yes	<0.0001
100:Simvastatin vs. 100:2	Yes	<0.0001	200:Simvastatin vs. 200:2	Yes	<0.0001
100:Simvastatin vs. 200:Simvastatin	Yes	<0.0001	200:Simvastatin vs. W/I	Yes	<0.0001
100:Simvastatin vs. 200: α -asarone	Yes	<0.0001	200:Simvastatin vs. 300: α -asarone	Yes	<0.0001
100:Simvastatin vs. 200:1	Yes	<0.0001	200:Simvastatin vs. 300:1	Yes	<0.0001
100:Simvastatin vs. 200:2	Yes	<0.0001	200:Simvastatin vs. 300:2	Yes	<0.0001
100:Simvastatin vs. 300:Simvastatin	Yes	<0.0001	200: α -asarone vs. 200:1	Yes	<0.0001
100:Simvastatin vs. 300: α -asarone	Yes	<0.0001	200:-asarone vs. 200:2	Yes	0.0002
100:Simvastatin vs. 300:1	Yes	<0.0001			
100:Simvastatin vs. 300:2	Yes	<0.0001	300:Simvastatin vs. 300: α -asarone	No	>0.9999
			300:Simvastatin vs. 300:1	Yes	<0.0001
			300:Simvastatin vs. 300:2	Yes	<0.0001

	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
10:α-asarone vs. W/I	Yes	<0.0001	50:α-asarone vs. W/I	Yes	<0.0001
10:α-asarone vs. DMSO	Yes	<0.0001	50:α-asarone vs. DMSO	Yes	<0.0001
10:α-asarone vs. 10:1	Yes	<0.0001	50:α-asarone vs. 50:1	Yes	<0.0001
10:α-asarone vs. 10:2	Yes	<0.0001	50:α-asarone vs. 50:2	Yes	<0.0001
10:α-asarone vs. 50:Simvastatin	Yes	<0.0001	50:α-asarone vs. 100:Simvastatin	Yes	<0.0001
10:α-asarone vs. 50:α-asarone	Yes	<0.0001	50:α-asarone vs. 100:α-asarone	Yes	<0.0001
10:α-asarone vs. 50:1	Yes	<0.0001	50:α-asarone vs. 100:1	Yes	<0.0001
10:α-asarone vs. 50:2	No	>0.9999	50:α-asarone vs. 100:2	No	>0.9999
10:α-asarone vs. 100:Simvastatin	No	0.0032	50:α-asarone vs. 200:Simvastatin	No	0.5467
10:α-asarone vs. 100:α-asarone	Yes	<0.0001	50:α-asarone vs. 200:α-asarone	Yes	<0.0001
10:α-asarone vs. 100:1	Yes	<0.0001	50:α-asarone vs. 200:1	Yes	<0.0001
10:α-asarone vs. 100:2	Yes	<0.0001	50:α-asarone vs. 200:2	Yes	<0.0001
10:α-asarone vs. 200:Simvastatin	Yes	<0.0001	50:α-asarone vs. 300:Simvastatin	Yes	<0.0001
10:α-asarone vs. 200:α-asarone	Yes	<0.0001	50:α-asarone vs. 300:α-asarone	Yes	<0.0001
10:α-asarone vs. 200:1	Yes	<0.0001	50:α-asarone vs. 300:1	Yes	<0.0001
10:α-asarone vs. 200:2	Yes	<0.0001	50:α-asarone vs. 300:2	Yes	<0.0001
10:α-asarone vs. 300:Simvastatin	Yes	<0.0001			
10:α-asarone vs. 300:α-asarone	Yes	<0.0001			
10:α-asarone vs. 300:1	Yes	<0.0001			

	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
100:α-asarone vs. W/I	Yes	<0.0001	200:α-asarone vs. W/I	Yes	<0.0001
100:α-asarone vs. DMSO	Yes	<0.0001	200:α-asarone vs. DMSO	Yes	<0.0001
100:α-asarone vs. 100:1	No	0.1159	200:α-asarone vs. 300:Simvastatin	Yes	<0.0001
100:α-asarone vs. 100:2	Yes	<0.0001	200:α-asarone vs. 300:α-asarone	Yes	<0.0001
100:α-asarone vs. 200:Simvastatin	Yes	<0.0001	200:α-asarone vs. 300:1	No	0.9983
100:α-asarone vs. 200:α-asarone	Yes	<0.0001	200:α-asarone vs. 300:2	No	0.012
100:α-asarone vs. 200:1	Yes	0.0008			
100:α-asarone vs. 200:2	Yes	<0.0001	300:α-asarone vs. 300:1	Yes	<0.0001
100:α-asarone vs. 300:Simvastatin	Yes	<0.0001	300:α-asarone vs. 300:2	Yes	<0.0001
100:α-asarone vs. 300:α-asarone	Yes	<0.0001			
100:α-asarone vs. 300:1	Yes	<0.0001			
100:α-asarone vs. 300:2	Yes	<0.0001			

	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
10:1 vs. W/I	Yes	<0.0001	50:1 vs. W/I	Yes	<0.0001
10:1 vs. DMSO	Yes	<0.0001	50:1 vs. DMSO	Yes	<0.0001
10:1 vs. 10:2	Yes	<0.0001	50:1 vs. 50:2	Yes	<0.0001
10:1 vs. 50:Simvastatin	Yes	<0.0001	50:1 vs. 100:Simvastatin	Yes	<0.0001
10:1 vs. 50:α-asarone	Yes	0.0002	50:1 vs. 100:α-asarone	Yes	0.0002
10:1 vs. 50:1	Yes	<0.0001	50:1 vs. 100:1	No	0.9669
10:1 vs. 50:2	Yes	<0.0001	50:1 vs. 100:2	Yes	<0.0001
10:1 vs. 100:Simvastatin	Yes	<0.0001	50:1 vs. 200:Simvastatin	Yes	<0.0001
10:1 vs. 100:α-asarone	Yes	<0.0001	50:1 vs. 200:α-asarone	Yes	<0.0001
10:1 vs. 100:1	Yes	<0.0001	50:1 vs. 200:1	Yes	<0.0001
10:1 vs. 100:2	Yes	<0.0001	50:1 vs. 200:2	Yes	<0.0001
10:1 vs. 200:Simvastatin	Yes	<0.0001	50:1 vs. 300:Simvastatin	Yes	<0.0001
10:1 vs. 200:α-asarone	Yes	<0.0001	50:1 vs. 300:α-asarone	Yes	<0.0001
10:1 vs. 200:1	Yes	<0.0001	50:1 vs. 300:1	Yes	<0.0001
10:1 vs. 200:2	Yes	<0.0001	50:1 vs. 300:2	Yes	<0.0001
10:1 vs. 300:Simvastatin	Yes	<0.0001			
10:1 vs. 300:α-asarone	Yes	<0.0001			
10:1 vs. 300:1	Yes	<0.0001			
10:1 vs. 300:2	Yes	<0.0001			

	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
100:1 vs. W/I	Yes	<0.0001	200:1 vs. W/I	Yes	<0.0001
100:1 vs. DMSO	Yes	<0.0001	200:1 vs. DMSO	Yes	<0.0001
100:1 vs. 100:2	Yes	<0.0001	200:1 vs. 200:2	No	>0.9999
100:1 vs. 200:Simvastatin	Yes	<0.0001	200:1 vs. 300:Simvastatin	Yes	<0.0001
100:1 vs. 200:α-asarone	Yes	<0.0001	200:1 vs. 300:α-asarone	Yes	<0.0001
100:1 vs. 200:1	Yes	<0.0001	200:1 vs. 300:1	Yes	<0.0001
100:1 vs. 200:2	Yes	<0.0001	200:1 vs. 300:2	Yes	<0.0001
100:1 vs. 300:Simvastatin	Yes	<0.0001			
100:1 vs. 300:α-asarone	Yes	<0.0001	300:1 vs. 300:2	No	0.5467
100:1 vs. 300:1	Yes	<0.0001			
100:1 vs. 300:2	Yes	<0.0001			

	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
10:2 vs. W/I	Yes	<0.0001	50:2 vs. W/I	Yes	<0.0001
10:2 vs. DMSO	Yes	<0.0001	50:2 vs. DMSO	Yes	<0.0001
10:2 vs. 50:Simvastatin	Yes	<0.0001	50:2 vs. 100:Simvastatin	Yes	0.0008
10:2 vs. 50:α-asarone	Yes	<0.0001	50:2 vs. 100:α-asarone	Yes	<0.0001
10:2 vs. 50:1	Yes	<0.0001	50:2 vs. 100:1	Yes	<0.0001
10:2 vs. 50:2	Yes	<0.0001	50:2 vs. 100:2	Yes	<0.0001
10:2 vs. 100:Simvastatin	No	0.2816	50:2 vs. 200:Simvastatin	Yes	<0.0001
10:2 vs. 100:α-asarone	Yes	<0.0001	50:2 vs. 200:α-asarone	Yes	<0.0001
10:2 vs. 100:1	Yes	<0.0001	50:2 vs. 200:1	Yes	<0.0001
10:2 vs. 100:2	Yes	<0.0001	50:2 vs. 200:2	Yes	<0.0001
10:2 vs. 200:Simvastatin	Yes	<0.0001	50:2 vs. 300:Simvastatin	Yes	<0.0001
10:2 vs. 200:α-asarone	Yes	<0.0001	50:2 vs. 300:α-asarone	Yes	<0.0001
10:2 vs. 200:1	Yes	<0.0001	50:2 vs. 300:1	Yes	<0.0001
10:2 vs. 200:2	Yes	<0.0001	50:2 vs. 300:2	Yes	<0.0001
10:2 vs. 300:Simvastatin	Yes	<0.0001			
10:2 vs. 300:α-asarone	Yes	<0.0001			
10:2 vs. 300:1	Yes	<0.0001			
10:2 vs. 300:2	Yes	<0.0001			

	<u>Significant?</u>	<u>Adjusted P Value</u>		<u>Significant?</u>	<u>Adjusted P Value</u>
100:2 vs. W/I	Yes	<0.0001	200:2 vs. W/I	Yes	<0.0001
100:2 vs. DMSO	Yes	<0.0001	200:2 vs. DMSO	Yes	<0.0001
100:2 vs. 200:Simvastatin	No	0.8191	200:2 vs. 300:Simvastatin	Yes	<0.0001
100:2 vs. 200:α-asarone	Yes	<0.0001	200:2 vs. 300:α-asarone	Yes	<0.0001
100:2 vs. 200:1	Yes	<0.0001	200:2 vs. 300:1	Yes	<0.0001
100:2 vs. 200:2	Yes	<0.0001	200:2 vs. 300:2	Yes	<0.0001
100:2 vs. 300:Simvastatin	Yes	<0.0001			
100:2 vs. 300:α-asarone	Yes	<0.0001			
100:2 vs. 300:1	Yes	<0.0001			
100:2 vs. 300:2	Yes	<0.0001			
