SUPPORTING INFORMATION

Identification of dual-target compounds with antifungal and anti-NLRP3 inflammasome activity

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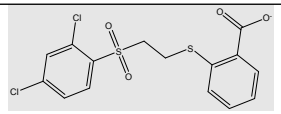
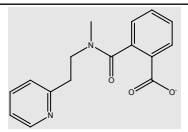
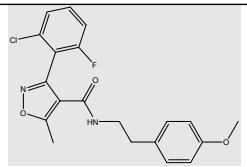
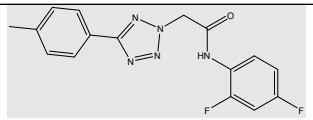
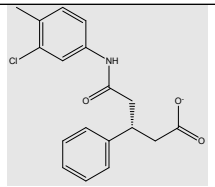
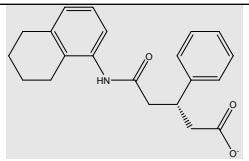
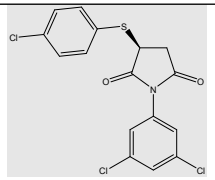
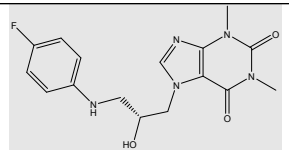
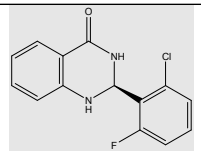
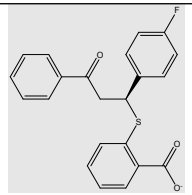
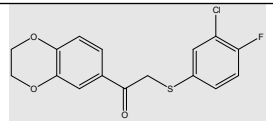
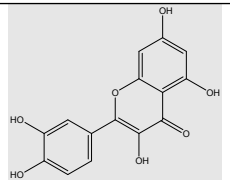
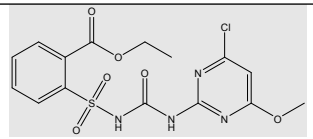
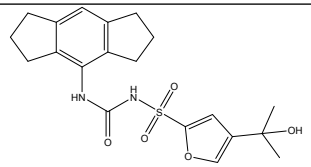
Table S1. Maybridge Compounds. Details on predicted actives compounds ordered and experimentally tested.

Cmpd #	Structure	SMILES	Vendor #	AHAS Docking Score	NLRP3 Docking Score	PAINS Filter	Aggregation Filter
1		c1cccc(c1C([O-])=O)SCCS(=O)(=O)c2c(Cl)cc(Cl)cc2	HTS02260	-9.79	-8.78	Passed	Passed
2		c1cccc(C([O-])=O)c1C(=O)N(C)CCc2ccccn2	HTS09182	-10.18	-8.52	Passed	Passed
3		COc(cc1)ccc1CCNC(=O)c2c(C)onc2-c3c(F)cccc3Cl	JFD01669	-9.89	-10.14	Passed	Passed
4		c1cc(F)cc(F)c1NC(=O)Cn(n2)nnc2-c(cc3)ccc3C	NRB00134	-9.70	-8.63	Passed	Passed
5		Cc1c(Cl)cc(cc1)NC(=O)CC(CC([O-])=O)c2ccccc2	PD00456	-9.47	-9.17	Passed	Passed
6		c1ccccc1C(CC([O-])=O)CC(=O)Nc2cccc(c23)CCCC3	PD00462	-10.07	-8.33	Passed	Passed
7		c1cc(Cl)ccc1SC(C2=O)CC(=O)N2c3cc(Cl)cc(Cl)c3	RJC03163	-9.83	-8.54	Passed	Passed

8		c1cc(F)ccc1NCC(O)Cn2cnc(c23)n(C)c(=O)n(C)c3=O	RJC03968	-9.381	-9.33	Passed	Passed
9		Clc1cccc(F)c1C(NC2=O)Nc(c23)cccc3	S12245	-9.45	-9.74	Passed	Passed
10		c1ccccc1C(=O)CC(c2ccc(F)cc2)Sc(c3C([O-])=O)cccc3	S15464	-10.19	-9.48	Passed	Passed
11		Fc1c(Cl)cc(cc1)SCC(=O)c(c2)ccc(c23)OCCO3	SPB08273	-9.38	-8.28	Passed	Passed
12		Oc1cc(O)cc(c12)oc(c(c2=O)O)-c3cc(O)c(O)cc3	XBX00307	-8.36	-8.36	Failed	Passed
Structures & Docking Scores for Known AHAS/NLRP3 Inhibitors							
13		CCOC(=O)c1c(cccc1)S(=O)(=O)NC(=O)Nc(n2)nc(Cl)cc2OC	C.E.	-6.06	-1.98	Passed	Passed
14		CC(C)(O)c1cc(oc1)S(=O)(=O)NC(=O)Nc2c(CCC3)c3cc(c24)CCC4	MCC950	N/A[a]	-7.64	Passed	Passed

[a]*No docking results could be generated for MCC950 in AHAS due to steric restrictions of the active site.*

Table S2. Strains used or created in this study.

Strain Name	***Parent***	***Species***	***Genotype***	***Reference***
SC5314	n/a	*C. albicans*	*reference*	1
[429]0832	n/a	*C. auris*	*wild-type*	1
CD36	n/a	*C. dubliniensis*	*reference*	1
CBS138	n/a	*C. glabrata*	*reference*	1
81-B-5	n/a	*C. krusei*	*wild-type*	1
CDC317	n/a	*C. parapsilosis*	*reference*	1
MYA3404	n/a	*C. tropicalis*	*reference*	1
BWP17	SC5314	*C. albicans*	*ILV2*/*ILV2* Δ/Δ*arg4* Δ/Δ*his1* Δ/Δ*ura3*	2
JM01	BWP17	*C. albicans*	ILV2/Δ*ilv2*::*ARG4* Δ/Δ*his1* Δ/Δ*ura3*	This study
JM02	JM01	*C. albicans*	*ILV2*/Δ*ilv2*::*ARG4* Δ/Δ*his1* Δ*ura3*/Δ*ura3*::*URA3*	This study
JM03	JM01	*C. albicans*	*ILV2*/Δ*ilv2*::*ARG4* Δ/Δ*his1* Δ*ura3*/Δ*ura3*::*URA3*-Pr*ACT1-ILV2*	This study
JM04	JM01	*C. albicans*	*ILV2*/Δ*ilv2*::*ARG4* Δ/Δ*his1* Δ*ura3*/Δ*ura3*::*URA3*-Pr*TEF1-ILV2*	This study
JM05	JM02	*C. albicans*	*ILV2*/Δ*ilv2*::*ARG4* Δ/Δ*his1*::*HIS1* Δ*ura3*/Δ*ura3*::*URA3*	This study
JM06	JM03	*C. albicans*	Δ*ilv2*::*HIS1*/Δilv2::*ARG4* Δ/Δ*his1* Δ*ura3*/Δ*ura3*::*URA3*-Pr*ACT1-ILV2*	This study
JM07	JM04	*C. albicans*	Δ*ilv2*::*HIS1*/Δilv2::*ARG4* Δ/Δ*his1* Δ*ura3*/Δ*ura3*::*URA3*-Pr*TEF1-ILV2*	This study

Table S3. Primers used in this study.

Primer name	Sequence[a] 5`→ 3`
ACT1PRSEQF	CACCAAGATTTATTGCCAACG
TEF1PRSEQF	TTTTTGCTGTTCACTTTCTCG
ADH13SEQR	ATATCGCACTCACGTAAACAC
ILV2ORF-F-SalI	TCA***GTCGAC***ATGATTTCTCGTAATTTGAGAACTTCATCAACG
ILV2ORF-R-MluI	TCA***ACGCGT***CTAATATTTACCACCAGTTCTTTCTTTTC
ILV2DISF	TTTGTTTTACTTCTTCTTCTTCTTTCTTCTTTACAGATTCAAACTATTAATTCAAACAAATGTTTTCCCAGTCACGACGTT
ILV2DISR	GACTTTTTTTTTTTTATAAAAAGAATAATAGATATACAAAACAAGAAGAAGAAGCCAATTTGGTAATGTGGAATTGTGAGCGGATA
ILV2AMPF	TCTGTCTAAATGCTACCAACTGGCC
ILV2AMPR	ATCACGTCTACTAAATCTTGGACTACGC
ARG4INTF	AAGCTAGTGTGGAAAGAAGAG
ARG4INTR	AATGACTGAATTATGTCGGTC
LUXINTDETF	CTGACCTTTAGTCTTTCCTGC
LUXINTDETR	CAGTAGTACTTGTTGTTGTATCG
HIS1DETF	TGCATTAATCTTCTTGCCTGC
HIS1INTR5	GGAGGATGAGGAGACAGAAGTTAGT
HIS1INTF	ACTGTATCCTCTTCTGTCCCC
HIS1INTR	CGACCATATGGGAGAGCTCCC
ILV2DETF	GCATTAATGGATGGGGTACCATTAGTGG
ILV2DETR	AGCAGCAGATCCATGCATACC
ILV2QPCR-R	ATTCTTCTTGGTAATTCCGCCAC
CaACT1QPCR-R	TTGGATTCTGGTGATGGTGTTA
CaACT1QPCR-R	TCAAGTCTCTACCAGCCAAATC

[a]*Underlined sequences denote complementarity to plasmids pGEMHIS1 and pRSARG4ΔSpe. Bold, italics sequences denote restriction enzyme sites.*

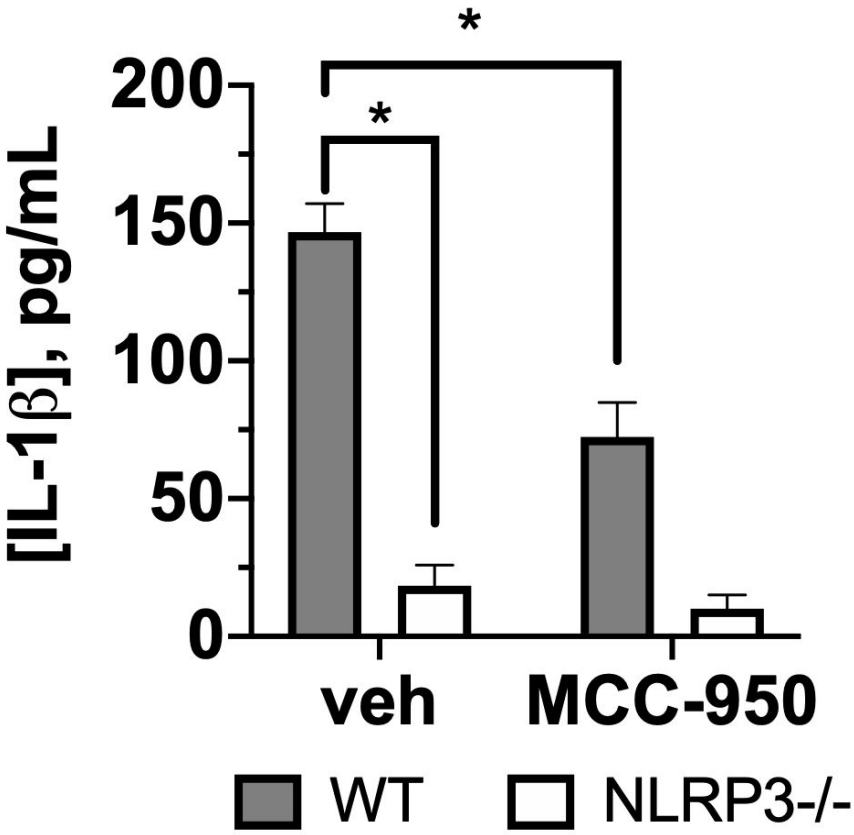


Figure S1. IL-1β release in THP1 cells is largely NLRP3-dependent. Differentiated WT or NLPR3-/- THP1 cells were treated with vehicle (0.5% DMSO) or 10 nM MCC-950 for 1 h prior to challenge with LPS (20 ng) for 3.5 h and ATP (5 mM) for 30 min. IL-1β release was quantified by ELISA and blank values subtracted from unstimulated controls. Data is the mean of independent experiments (n=3) conducted in technical quadruplicate. Data was analyzed using a one-way ANOVA and Tukey's post-test. *, $p < 0.05$.

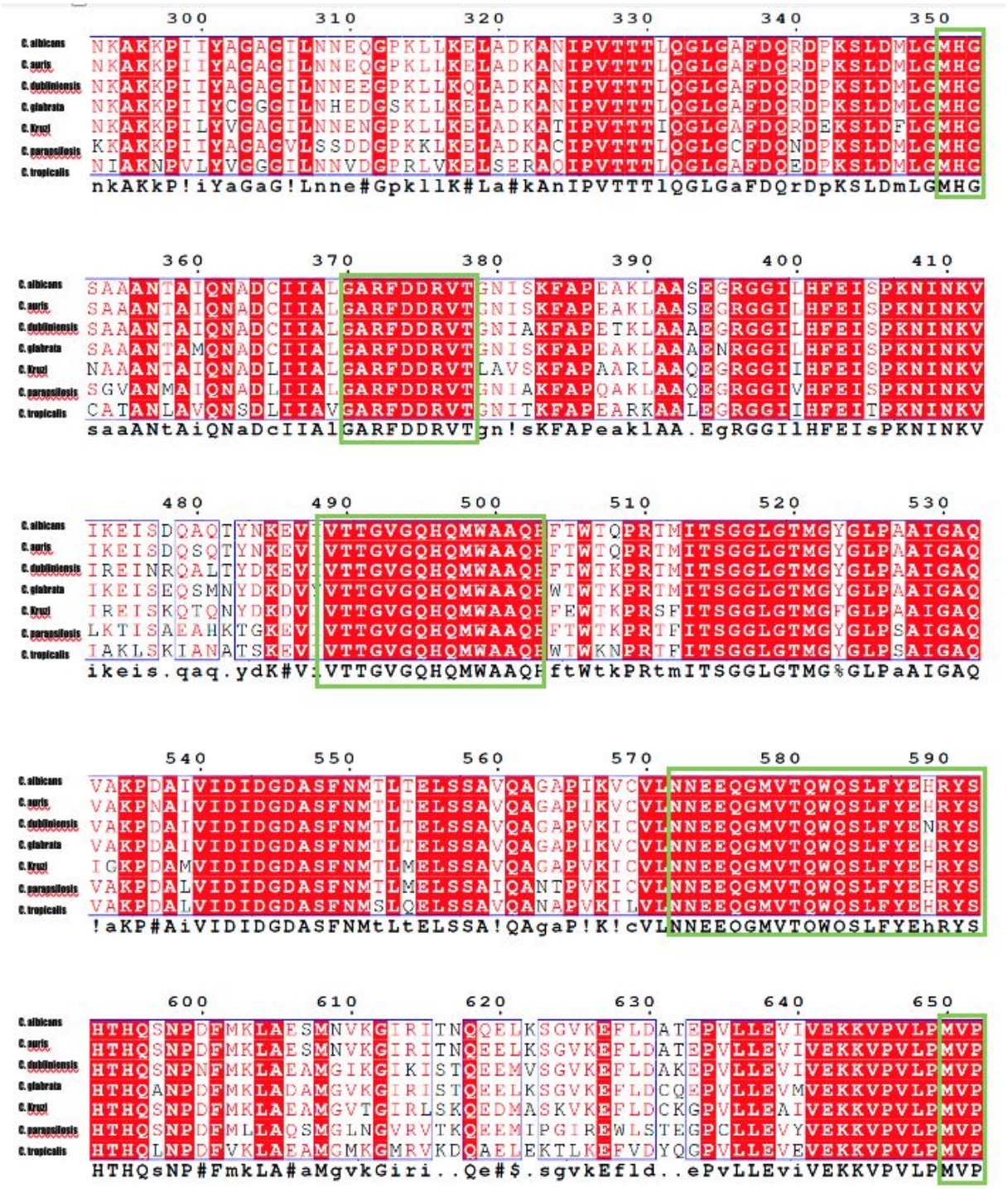


Figure S2. Alignment of AHAS orthologs from several *Candida* species. ClustalW was used to generate alignments of the AHAS protein from several clinically relevant Candida species and shows a high degree of sequence conservation, with near 100% identity of active site residues (boxed in green). The sole outlier being *C. dubliniensis* H589N.

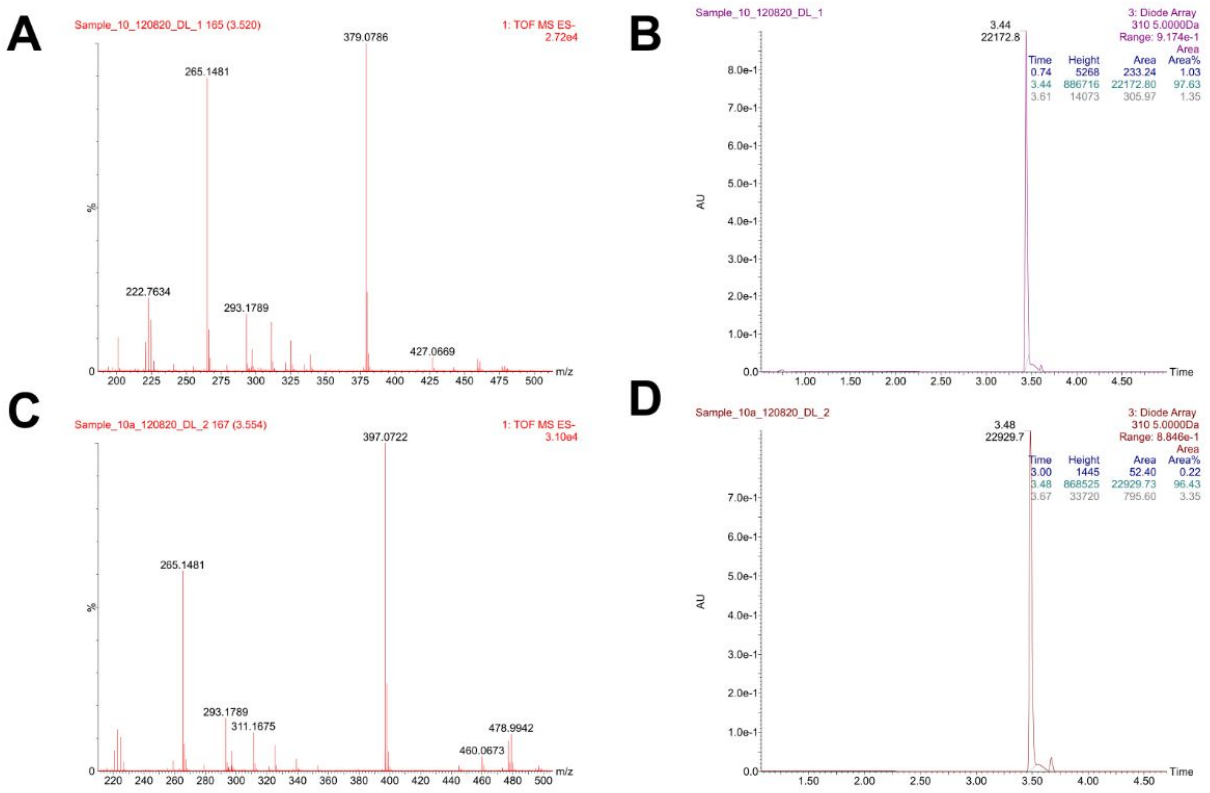


Figure S3. LC/MS confirmation of purity/identify of lead compounds 10 and 10a. (A) Chromatogram of compound **10** using photo diode array detection. **(B)** Time of flight mass spectrum of compound **10** using negative ionization mode. **(C)** Chromatogram of compound **10a** using photo diode array detection. **(D)** Time of flight mass spectrum of compound **10a** using negative ionization mode.

Supporting PDB structures submitted as separate attachments:

A. *SupportingA_MCC950_NLRP3_CoStructure.pdb*
B. *SupportingB_Compound10_NLRP3_CoStructure.pdb*
C. *SupportingC_Compound10_AHAS_CoStructure.pdb*

References

1. Willems, H. M. E.; Lowes, D. J.; Barker, K. S.; Palmer, G. E.; Peters, B. M. Comparative Analysis of the Capacity of the *Candida* Species To Elicit Vaginal Immunopathology. *Infect Immun* **2018,** 86, e00527-18.
2. Wilson, R. B.; Davis, D.; Mitchell, A. P. Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. *J Bacteriol* **1999,** 181, 1868-1874.