SUPPORTING INFORMATION

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

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Cmpd #	Structure	SMILES	Vendor #	AHAS	NLRP3	PAINS	Aggregation
				Docking	Docking	Filter	Filter
	0.			Score	Score		
1	c1cccc(c1C([O-])=O)SCCS(=O)(=O)c2c(CI)cc(CI)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)c2cccc2	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

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

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 S		-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		-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/Aª	-7.64	Passed	Passed

^aNo 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/Δilv2::ARG4 Δ/Δhis1 Δura3/Δura3::URA3	This study
JM03	JM01	C. albicans	ILV2/Δilv2::ARG4 Δ/Δhis1 Δura3/Δura3::URA3-PrACT1-ILV2	This study
JM04	JM01	C. albicans	ILV2/Δilv2::ARG4 Δ/Δhis1 Δura3/Δura3::URA3-PrTEF1-ILV2	This study
JM05	JM02	C. albicans	ILV2/Δilv2::ARG4 Δ/Δhis1::HIS1 Δura3/Δura3::URA3	This study
JM06	JM03	C. albicans	Δilv2::HIS1/Δilv2::ARG4 Δ/Δhis1 Δura3/Δura3::URA3-PrACT1-ILV2	This study
JM07	JM04	C. albicans	Δilv2::HIS1/Δilv2::ARG4 Δ/Δhis1 Δura3/Δura3::URA3-PrTEF1-ILV2	This study

Table S3. Primers used in this study.

Primer name	Sequence ^a 5`→ 3`
ACT1PRSEQF	CACCAAGATTTATTGCCAACG
TEF1PRSEQF	TTTTTGCTGTTCACTTTCTCG
ADH13SEQR	ATATCGCACTCACGTAAACAC
ILV2ORF-F-Sall	TCA GTCGAC ATGATTTCTCGTAATTTGAGAACTTCATCAACG
ILV2ORF-R-Mlul	TCA ACGCGT CTAATATTTACCACCAGTTCTTTCTTTC
ILV2DISF	TTTGTTTTACTTCTTCTTCTTCTTCTTCTTCTTACAGATTCAAACTATTAATTCAAACAAA
ILV2DISR	GACTTTTTTTTTTTATAAAAAGAATAATAGATATACAAAACAAGAAGAAGAAGAAGCCAATTTGGTAA <u>TGTGGAATTGTGAGCGGATA</u>
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

^aUnderlined sequences denote complementarity to plasmids pGEMHIS1 and pRSARG4ΔSpe. Bold, italics sequences denote restriction enzyme sites.



Figure S1. IL-1 β 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-1 β 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.

-	300	310	320	330	340	350
e albisant			T THE T P HAN	TOTOMOUT		
C aurie	NKAKKPIIIAGAG	JILNNEQGPKL	LKELADKAN	TPVTTTLQGL	GAFDORDPKS	LDMLGMHG
C dubliniansis	NEAREDITEAGAC	TINNEROPEL	LYOTADKAN	TRUTTINGL	CARDODDEKS	LDMLGMHG
S. Bolobasia	NKAKKDITYCCC	TINUEDCCKI	LKELADKAN	TRUTTINGL	CAPDOPDEKS	LDMLGMHG
6. glaorata	NKAKKDTLYVCA	TINNENCORI	LKETADKAT	TRVTTTTOCL	CAPDORDEKS	
e. Mill	KKAKKDITVACA	VISSDDCPKK	LKELADKAC	TPVTTTLOCL	CEDONDEKS	LDMLCMHC
C. DALADSHOSIS	NTAKNPVLYVGG	TINNVDGPRL	VKELSERAC	TPVTTTLOGL	GAFDOEDPKS	LDMLGMHG
C. tropicalis	nkAKkP! iYaGa(Lnne#Gpkl	1K#La#kAn	IPVTTT10GL	GaFDOrDpKS	LDmLGMHG
					ourberbpho	20 m2 onio
	360	370	380	390	400	410
C. albicans	SAAANTAIQNADO	IIALGARFDD	RVT GNISKF	APEAKLAASE	GRGGILHFEI	SPKNINKV
C. auris	SAAANTAIONADO	IIAL GARFDD	RVTGNISKE	APEAKLAASE	GRGGILHFEI	SPKNINKV
C. dubliniensis	SAAANTAIQNADO	IIAL GARFDD	RVTGNIAKF	APETKLAAAE	GRGGILHFEI	SPKNINKV
C. glabrata	SAAANTAMQNADC	IIALGARFDD	RVTGNISKE	APEAKLAAAE	NRGGILHFEI	SPKNINKV
C. Kruzi	NAAANTAIQNADI	IIAL GARFDD	RVTLAVSKF	APAARLAAQE	GRGGI <mark>I</mark> HFEI	SPKNINKV
C. paraesilosis	SGVANMAIQNADI	IIALGARFDD	RVT GNIAKF	AP QAKL <mark>AA</mark> QE	G <mark>RGGI</mark> VHFEI	SPKNINKV
C. tropicalis	CATANLAVQNSDI	IIAVGARFDD	RVT GNITKF	APEARKAALE	GRGGI <mark>I</mark> HFEI	TPKNINKV
	saaANtAiQNaDo	IIALGARFDD	RVTgn!sKF	APeaklAA.E	gRGGIlHFEI	SPKNINKV
	480	490	500	510	520	530
E siblesos	THERE					
6. divisions A garrie	IKEISDQAQTYNK	EV VTTGVGQ	HQMWAAQHF	TWIOPRIMIT	SGGLGTMGYG	LPAAIGAQ
e dubliniansis	IKEISDQSQTYNK	EV VTTGVGQ	HOWWAAOH	TWICPRIMIT	SGGLGTMGYG	LPMAIGAQ
C. alabasis	IKEINKVALIIDK	EVI VTTGVGQ	HOMWAAQFE	TWIKPRIMIT	SGGLGTMGIG	LPAAIGAQ
e. grant na	IREISEQSMNIDE	DV VIIGVGQ	HOMWAAQEW	TWINPRIMIT.	SGGLGIMGIG	LPAAIGAQ
	LKTISAFAHKTGK	EV VTTGVGQ	HOMWAAOHE	TWTKPRTFTT	SCGLGTMGYG	LPSATGAO
C. Darapsilosis	TAKLSKTANATSK	EV VTTGVGQ	HOMWAAOHW	TWKNPRTFTT	SCGLGTMGYC	LPSATGAO
C. Tropicalis	ikeis gag vdK	#V VTTGVGO	HOMWAAOH	tWtkPRtmTT	SCGLGTMC&C	LPAATGAO
	Incio. ded. Jan		ngammer r	en en a nem a a	ood dormo oo	Di oni ong
	540	550	560	570	580	590
P albieans						
C auris	VARPDAIVIDIDG	DASFNMILIE	LSSAVQAGA	PIKVCVLNNE.	EQGMVTQWQS	LFYEHRYS
e dubliniansis	VAKPNAIVIDIDG	DASFNMTLTE	LSSAVQAGA	PIKVCVLNNE.	EQGMVTQWQS	LEVENDYS
C. alabrata	VARPDAIVIDIDO	DASENMILIE DASENMIT	LSSAVQAGA	PUKICVLNNE.	ROGMVTQWQS	LEVEURYC
C. Knut	TCKPDAMVTDTDC	DASENMELTE	LSSAVQAGA	DVKTOVINNE.	ROGMVTQWQS	LEVEUDVS
- COOL	VAKPDALVIDIDO	DASENMELME	LSSATOANT	DWKICVLNNE	FOGMUTOWOS	LEVENDVS
s. parapsilosis	VAKPDALVIDIDO	DASENMSLOE	LSSAVOANA	PVKTLVLNNE	ROGMVTOWOS	LEYEHRYS
e. tropicalis	!aKP#AiVIDIDG	DASENMLLLE	LSSA! OAga	PIKICVLNNE	EOGMVTOWOS	LEYEDRYS
	600	610	620	630	640	650
C. albicans	HTHOSNPDFMKLA	A E SMNVKGIRI	TNOQELKSO	VKEFLDATEP	VLLEVIVEKK	VPVLPMVP
C. auris	HTHQSNPDFMKLA	AESMNVKGIRI	TNOEELKSC	VKEFLDATEP	VLLEVIVEKK	VPVLPMVP
C. dubliniensis	HTHQSNPNFMKL2	AEAMGIKGIKI	STOEEMVSO	VKEFLDAKEP	VLLEVIVEKK	VPVLPMVP
C. glabrata	HTHQANPDFMKLZ	ADA <mark>M</mark> GVK <mark>G</mark> IRI	STQEELKSO	VKEFLDCQEP	VLLEVMVEKK	VPVLPMVP
C. Krugi	HTHQSNPDFMKL	AEA <mark>M</mark> GV TG IRL	SKQEDMASE	(VKEFLDCKGP	VLLEAIVEKK	VPVLPMVP
C. parapsilosis	HTHQSNPDFMLL2	A Q S M G L N <mark>G</mark> V R V	TKQEEMIPO	SIR <mark>E</mark> WLSTEG <mark>P</mark>	C <mark>lle</mark> VY <mark>VEKK</mark>	VPVLPMVP
C. tropicalis	HTHQLNPDFVKLA	AEAMGMK <mark>G</mark> MRV	KDQAELEKI	LKEFVDYQGP	VLLEVEVEKK	VPVLPMVP
	HTHQsNP#FmkLA	A#aMgvkGiri	Qe#\$.sc	vkEfldeP	VLLEVIVEKK	VPVLPMVP

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

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- 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.