

Figure S1: Structures azole drugs and agrochemicals. (A) Imidazole and 1,2,4 triazole substituents. (B) Sterol 14α -demethylase inhibitors used as triazole drugs. (C) Sterol 14α -demethylase inhibitors used as fungicides as agrochemicals. Fluconazole, voriconazole, propiconazole, tebuconazole, epoxiconazole and triadimenol are short-tailed azole drugs. Isavuconazole is a mid-tailed azole drug. Itraconazole and posaconazole are long-tailed azole drugs. Structures were modified from https://www.sigmaaldrich.com, 27.10.2020

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Genus	Species	I ype of Isolate	change	Quality wt	change	2 nd Mutation	IK	FLC	VRC	TDM*	IVU	ITC	PSC	AMB	strains	Reference
Ajellomyces	capsulatus	environmental	Y136F	polar	Non polar			n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[28]
Aspergillus	fumigatus	clinical	Y121F	polar	non polar	T289A	TR46	n.a.	4->16	n.a.	16->16	0.5->16	0.25-1	n.a.	17	[32-35]
Aspergillus	fumigatus	environmental	Y121F	polar	non polar	T289A	TR46	n.a.	0.25->16	n.a.	0.125->8	0.25->16	0.06-1	n.a.	112	[136,142-144]
Aspergillus	fumigatus	environmental	Y121F	polar	non polar			n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[78]
Aspergillus	fumigatus	mutants	I301	polar	non polar			20	0.12	n.a.	n.a.	0.06	0.25	0.5	1	[36]
Candida	albicans	mutants	Y132F	polar	non polar			8	0.5	n.a.	n.a.	0.25	< 0.03	n.a.	1	[16]
Candida	albicans	mutants	Y132H	uncharged	+ charge			2	0.25	n.a.	n.a.	0.25	< 0.03	n.a.	1	[16]
Candida	auris	clinical	Y132F	polar	non polar			64->256	0.094-1.5	n.a.	n.a.	n.a.	n.a.	n.a.	12	[96]
Candida	auris	clinical	K143R	one N atom	3 N atoms			>256	2-4	n.a.	n.a.	n.a.	n.a.	n.a.	24	[96]
Candida	parapsilosis	clinical	Y132F	polar	non polar			8-256	0.12-2	n.a.	n.a.	n.a.	n.a.	n.a.	79	[27,46]
Candida	parapsilosis	clinical	Y132F	polar	non polar	R398I		64-128		n.a.	n.a.	n.a.	n.a.	n.a.	9	[46]
Candida	tropicalis	clinical	K143R	one N atom	3 N atoms			>64	8	n.a.	n.a.	1	1	n.a.	1	[124]
Cryptococcus	neoformans	mutants	Y145F	polar	non polar			128	2-3	n.a.	n.a.	0.094-0.38	0.125-0.25	n.a.	1	[44]
Cryptococcus	neoformans	mutants	Y145F	polar	non polar	P6S, C45G, Q50L, S460T		128	2	n.a.	n.a.	0.38	0.25	n.a.	2	[44]
Mucor	circinelloides	n.a.	F129	non polar	-	A291		>64	16->16	n.a.	n.a.	n.a.	n.a.	n.a.	18	[49]
Mucor	circinelloides	clinical	F129	non polar	-	A291		64	8->16	n.a.	0.125->16	0.25-16	0.015->16	0.03-0.06	20	[145,146]
Rhizopus	arrhizus	n.a.	F129	non polar	-	A291		>64	4-16	n.a.	n.a.	n.a.	n.a.	n.a.	17	[49]
Rhizopus	arrhizus	clinical	F129	non polar	-	A291		64->64	4->16	n.a.	0.5-8	0.125-16	0.125->16	0.03-0.125	21	[145,146]
Rhizopus	microsporus	n.a.	F129	non polar	-	A291		n.a.	4-16	n.a.	n.a.	n.a.	n.a.	n.a.	13	[49]
Rhizopus	microsporus	clinical	F129	non polar	-	A291		n.a.	16->16	n.a.	1-8	n.a.	0.5->16	n.a.	26	[145,146]
Rhizopus	microsporus	clinical	F129	non polar	-	A291		64	1-16	n.a.	0.125-4	0.06-16	0.06-4	0.03-1	17	[146]
Scedosporium	apiospermum	clinical	Y136F	polar	non polar			n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	2	[50]
Blumeria	graminis	environmental	Y136F	polar	non polar			n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[51,52,119]
Mycosphaerella	graminicola	environmental	Y137F	polar	non polar			n.a.	n.a.	14.73	n.a.	n.a.	n.a.	n.a.	4	[52]
Parastagonospora	nodorum	environmental	Y144F	polar	non polar			n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[54]
Puccinia	triticina	environmental	Y134F	polar	non polar			n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[55]
Uncinula	necator	environmental	Y136F	polar	non polar			n.a.	n.a.	8.2-22.1	n.a.	n.a.	n.a.	n.a.	n.a.	[56,147]

Table S1: Sterol 14α -demethylase amino acid substitutions and their phenotypic effects.

Legend: not available (n.a.), amphotericin B (AMB), fluconazole (FLC), itraconazole (ITC), posaconazole (PCZ), voriconazole (VCZ), isavucoanzole (IVU), tandem repeat in the promotor region of the lanosterol 14 alpha demethylase gene (TR), chemical character of wild type amino acid (quality of wt), amino acid (AA), phenylalanine (F), tyrosine (Y), threonine (T), isoleucine (I), arginine (R), lysine (K).

Table S2: EUCAST Clinical Brea	akpoints (CBPs) of human	pathogenic fungi.
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	AMB	FLC	ITC	PCZ	VCZ	IVU
	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)
Aspergillus flavus	n.a.	n.a.	1	n.a.	n.a.	2
Aspergillus fumigatus	1	n.a.	1	0.25	1	2
Aspergillus nidulans	n.a.	n.a.	1	n.a.	1	0.25
Aspergillus niger	1	n.a.	n.a.	n.a.	n.a.	n.a.
Aspergillus terreus	n.a.	n.a.	1	0.25	n.a.	1
Candida albicans	1	4	0.06	0.06	0.25	n.a.
Candida tropicalis	1	4	0.125	0.06	0.25	n.a.

Legend

Not available (n.a.), amphotericin B (AMB), fluconazole (FLC), itraconazole (ITC), posaconazole (PCZ), voriconazole (VCZ), isavuconazole (IVU)

Clinical Breakpoints according to EUCAST Antifungal Clinical Breakpoint Table v. 10.0 valid from 2020-02-04

Isolates expressing higher minimal inhibitory concentrations (MIC) than listed in the table above are considered resistant, exhibiting a lower or the exact MIC than given in the table above are considered susceptible.

Table S3: Itraconazole and lanosterol binding plus innate resistance in eukaryotic CYP51s. Residues within 4 Å of itraconazole in *S. cerevisiae* SDM are shown in bold. Residues within 4 Ű of lanosterol in the human CYP51 D231A H314A mutant that has high substrate occupancy are highlighted in green (in general, possible hydrophobic interactions). Residues with their main chain nitrogen possibly involved in a water-mediated hydrogen bond network with the hydroxyl of lanosterol (M378, M487) are highlighted in gray while the residue (I379) using its main chain carbonyl to make a direct hydrogen bond with lanosterol is highlighted in gray and in **bold**. Some residues were where alignments and some experimental work indicate possible roles in substrate specificity and/or as causes of innate resistance to azole drugs are highlight in light blue. Selected residues identified as relevant to innate azole resistance representative of molds are illustrated using *A. fumigatus* and for mucormycetes they are illustrated using *R. arrhizus*.

Helix B-loop-Helix C (SRS1)

- S. cerevisiae LDM 124 AAYAHLTTPVFGKGVIYDCP
- C. albicans LDM
- 116 DAYKHLTTPVFGKGVIYDCP
- A. fumigatus CYP51A 105 EVYSPLTTPVFGSDVVYDCP
- A. fumigatus CYP51B 120 EVYSPLTTPVFGRHVVYDCP
- *R. arrhizus* LDM F1
- 110 DAYNHMTKHVFGPEVVYDAP 112 AAYNHMTKYVFGNDIVFDTA
- R. arrhizus LDM F5
- H. sapiens CYP51 129 DVYSRLTTPVFGKGVAYDVP

Helix C

S.	<i>cerevisiae</i> LDM		147 LMEQKKFVK
C.	<i>albicans</i> LDM	139	LMEQKKFAK
Α.	<i>fumigatus</i> CYP51A	128	LMEQKKFIK
Α.	<i>fumigatus</i> CYP51B	143	LMEQKKFIK
R .	<i>arrhizus</i> LDM F1		136 FMEQKKFIK
R .	<i>arrhizus</i> LDM F5		136 FMEQKRFIK
H.	sapiens CYP51	152	FLEQKKMLK
T .	<i>aestivum</i> CYP51	1	31 RQEQFRFFT

F-F"

S.	cerevisiae LDM		236 F-TPINF
C.	<i>albicans</i> LDM	228	F-TPINF
Α.	<i>fumigatus</i> CYP51A	214	F-TPINF
Α.	<i>fumigatus</i> CYP51B	229	F-APINF
R .	<i>arrhizus</i> LDM F1		217 F-KPINF
R.	<i>arrhizus</i> LDM F5		217 F-RPINF
H.	sapiens CYP51	234	FSHAAWL
T .	<i>aestivum</i> CYP51		213 M-LPISV

Helix I (SRS3)

S.	cerevisiae LDM		306 NLLI GV LM G GQH T SAAT
С.	<i>albicans</i> LDM	299	NLLIGILMGGQHTSAST
Α.	<i>fumigatus</i> CYP51A	285	HMMITLLMAGQHSSSSI
Α.	<i>fumigatus</i> CYP51B	299	HMMIALLMAGQHSSSST
R.	<i>arrhizus</i> LDM F1		286 GMMIAVLFGGQHTSSTT
R.	<i>arrhizus</i> LDM F5		286 GILTAALFGGQHTSSTT
H.	<i>sapiens</i> CYP51	303	GMLIGLLLAGQHTSSTT
T .	<i>aestivum</i> CYP51		281 GLLIAALFAGQHTSSIT

K-K' loop S. cerevisiae LDM 379 P**LHS**LFR C. albicans LDM 299 PLHSIFR A. fumigatus CYP51A 363 SIHSIMR A. fumigatus CYP51B 372 PIHSIIR *R. arrhizus* LDM F1 347 PIFQMMR *R. arrhizus* LDM F5 347 PIFNMR H. sapiens CYP51 376 PIMIMMR T. aestivum CYP51 354 PLIMLLR C-terminal region S. cerevisiae LDM 505 DFTS-MVTL C. albicans LDM 504 DYSS-MVVL A. fumigatus CYP51A 490 DYSS-LFSG A. fumigatus CYP51B 499 DYSS-LFSK R. arrhizus LDM F1 478 DYTS-MVVV *R. arrhizus* LDM F5 476 DYTS-MVVV H. sapiens CYP51 483 NYTT-MIHT T. aestivum CYP51 467 NDWNAMVVG

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