From our lab to a European library: bioprofiling our compounds through EU-OPENSCREEN

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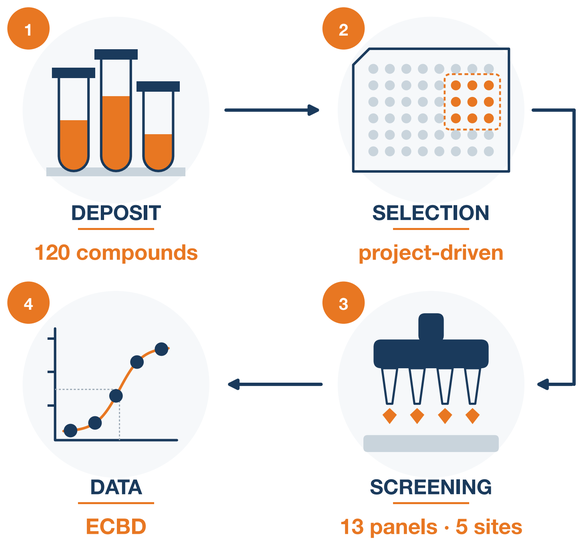
WHY THIS SERIES, WHY NOW

Invasive infections caused by *Candida auris*, *C. albicans* and *Aspergillus fumigatus* are a growing clinical threat, and rising azole resistance keeps closing off the options that remain. What is needed is not another analogue of the existing classes, but chemically diverse antifungal scaffolds.

EU-OPENSCREEN lets an academic group deposit its library into a pan-European infrastructure and receive systematic bioprofiling across diverse targets and organisms — a scale of characterisation rarely within reach of a single lab.

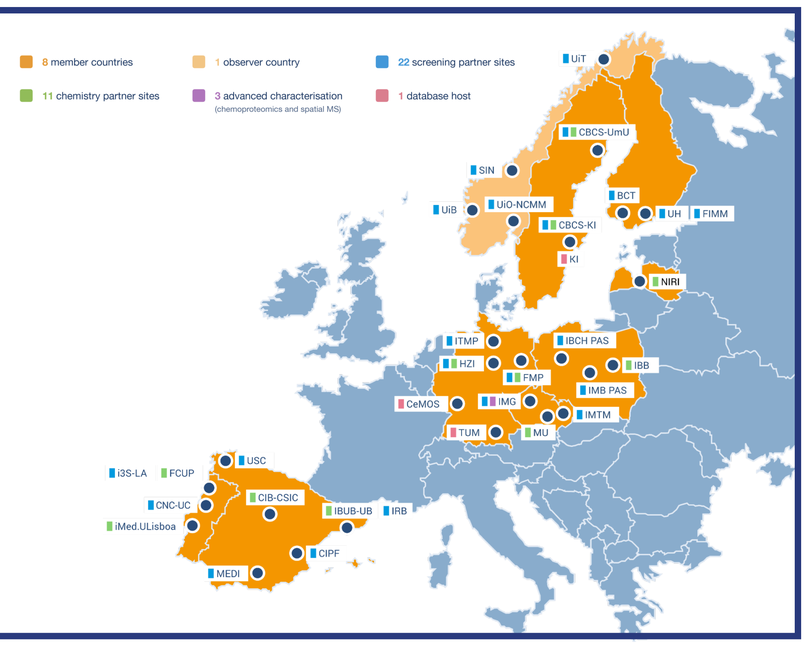
First results for our contribution to the Chemical Academic Library: a 120-compound 3-arylcoumarin series, profiled in 13 assay exports at five partner sites.

Chemically homogeneous and drug-like.
All 120 share the coumarin core: MW 333 ± 56, cLog*P* 3.6 ± 0.6, and 119 of 120 carry zero Lipinski violations.



WHAT WAS SCREENED

Fundación MEDINA MSSA, *E. faecalis*, *C. albicans*, *C. auris*, *A. fumigatus* — 50 µM, 2 replicates	PRIMARY	hit ≥ 70 %
HZI Chemical Biology *E. coli*, *P. aeruginosa*, *A. baumannii*, *K. pneumoniae* — 50 µM, single point	PRIMARY	hit ≥ 50 %
EACL B01-06 Firefly / *Renilla* / NanoLuc luciferase inhibition	COUNTER	10 µM + dose–response
EACL B01-02 Redox: resazurin/DTT, resazurin/TCEP, phenol red oxidation	COUNTER	single concentration
USC BioFarma Kinetic solubility by nephelometry, 10 µM	PROPERTY	HIGH / MEDIUM / LOW
Target-based SARS-CoV-2 Nsp13 helicase; RAGE(VC1)–S100B disruption	TARGET	% inhibition



WHAT THE SCREENS REVEALED

120
compounds deposited and bioprofiled

13 × 5
assay panels across EU-OPENSCREEN sites

10
pan-antifungal hits ≥ 70 % vs all three fungi

2
clean hits, no flag in six interference screens

0 / 120
Gram-negative actives across three species

THE TRIAGE, AT A GLANCE

	C. auris	*C. albicans*	*A. fumigatus*	MSSA	Solubility	Luciferase
MJM561	**93**	**98**	**82**	42	high	clean
MJM560	**84**	**93**	**92**	33	high	clean
MJM562B	**98**	**99**	**106**	**100**	high	flag ×2
MJM452	**98**	**98**	**104**	19	high	flag ×1
MJM452B	**96**	**97**	**105**	13	high	flag ×1
MJM586	**92**	**83**	**72**	13	high	flag ×1
MJM565B	**85**	**92**	**71**	-3	high	flag ×1
MJM585B	**93**	**97**	**72**	12	high	flag ×2
MJM471	**96**	**98**	**80**	45	high	flag ×2
MJM619B	**95**	**94**	**77**	23	medium	flag ×2
MJM638	**96**	**90**	15	**91**	high	clean
MJM584B	**89**	**96**	56	**91**	high	flag ×1
MJM639	**95**	**91**	26	**101**	high	clean
MJM566	**92**	**92**	15	**80**	medium	clean
MJM559	**92**	**92**	-3	**74**	low	flag ×1
MJMJLG3	**92**	**97**	63	5	high	clean
MJM459	**93**	36	**72**	4	high	flag ×1
MJMJLG12B	**89**	**91**	57	0	high	flag ×2
MJM565	**85**	**90**	53	-1	high	flag ×2
MJM558B	**94**	**89**	64	32	medium	flag ×2
MJM624	**94**	**86**	64	12	medium	flag ×2
MJM551	**90**	**92**	49	5	low	flag ×2

% growth inhibition at 50 µM
−50 0 50 70 100
bold value = hit (≥ 70 %)

Figure 1. Growth inhibition at 50 µM for the 22 tiered candidates across four pathogens (left), with luciferase / redox interference flags and kinetic-solubility class (right). Mean of 2 replicates, Fundación MEDINA primary screens. Values above 100 % are complete inhibition within assay noise. All 22 compounds are clean in the three redox counter-screens. Tiers: **T1** clean pan-antifungal • **T2** pan-antifungal with a counter-screen or solubility caveat • **T3** antifungal + anti-MSSA • **T4** two-species antifungal.

STRUCTURE–ACTIVITY ANALYSIS

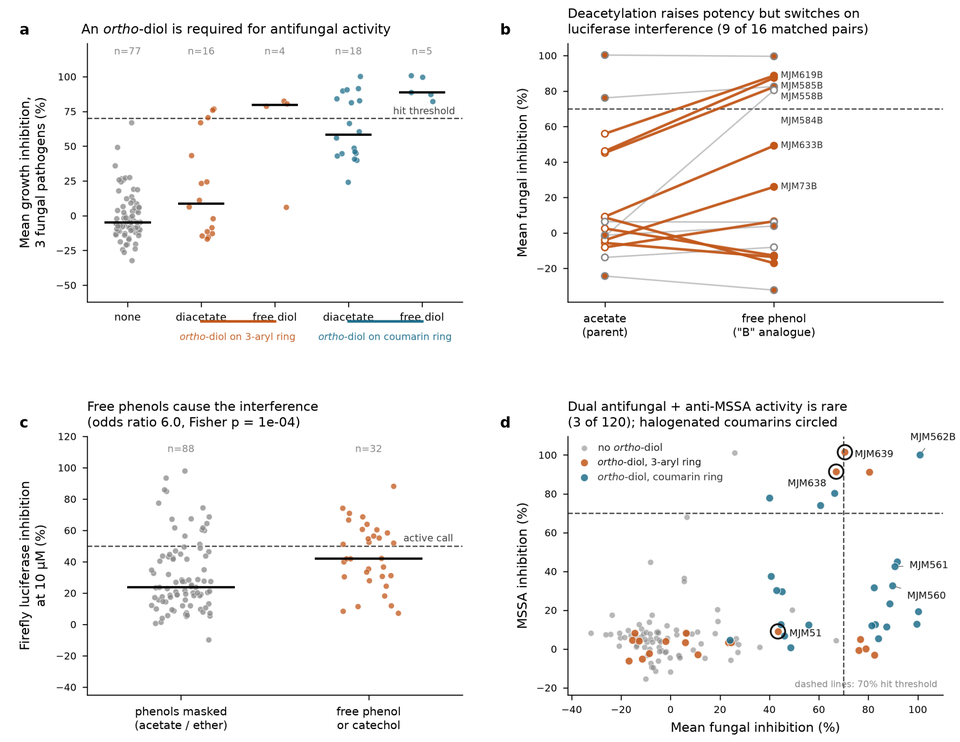
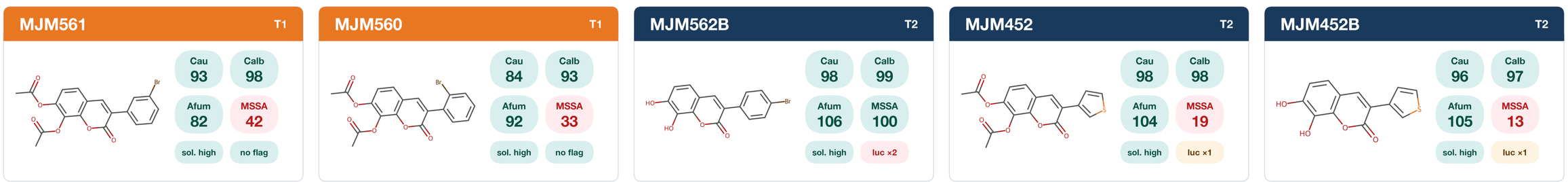


Figure 2. (a) Mean inhibition across the three fungi by *ortho*-diol class. (b) Matched acetate-to-free-phenol pairs; orange traces mark pairs where deacetylation switches on firefly-luciferase inhibition. (c) Firefly-luciferase inhibition at 10 µM by phenolic state. (d) Antifungal *versus* anti-MSSA activity, all 120 compounds; circles mark the three *ortho*-diols halogenated on the coumarin ring. Black bars, medians; dashed lines, hit thresholds.

THE MOLECULES



% growth inhibition at 50 µM (Fundación MEDINA, mean of 2 replicates); hit threshold 70 %. Cau Candida auris Calb C. albicans Afum Aspergillus fumigatus MSSA methicillin-sensitive Staphylococcus aureus.

Figure 3. The five highest-scoring 3-arylcoumarins, annotated with % inhibition at 50 µM against *C. auris* (Cau), *C. albicans* (Calb), *A. fumigatus* (Afum) and MSSA, and with the number of luciferase counter-screens flagged. **MJM561** and **MJM560** (left) are the two Tier-1 compounds: pan-antifungal with no flag in any of the six interference assays.

TWO HITS. ONE DIRECTION. WHAT COMES NEXT?

THE MOTIF
10 / 10
pan-antifungal hits carry an *ortho*-diol
43 of 120 compounds carry a catechol or *O*-acetyl catechol. Of the 77 without it, 73 don't present any relevant activity.

POSITION DECIDES
79.7 *vs* 8.7 %
3-aryl substituted free diol *vs* its diacetate
On the coumarin ring the motif works free or masked. On the 3-aryl ring only the free diol works.

MASKING DECOUPLES
25 / 32
phenolics flag firefly luciferase
Against 33 of 88 without a free phenol. The diacetate keeps the potency and removes the interference — the design rule.

TWO CLEAN HITS
MJM561 · MJM560
clean in all six interference counter-screens
82–98 % across the three fungi, HIGH solubility.
A selectivity index is still to be measured.

Hit threshold 70 % growth inhibition at 50 µM (Fundación MEDINA, mean of 2 replicates). Firefly-luciferase enrichment in phenolics: OR 6.0, Fisher exact $p = 1.5 \times 10^{-4}$. Halogenation of the coumarin ring adds an *S. aureus* arm, on n = 3.

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