Discovery of a Novel Dibromoquinoline Compound Exhibiting Potent Antifungal and Antivirulence Activity that Targets Metal ion Homeostasis

Haroon Mohammad[1,‡], Nehal H. Elghazawy[2,‡], Hassan Eldesouky[1,‡], Youssef A. Hegazy[1], Waleed Younis[1], Larisa Avrimova[3], Tony Hazbun[3,4], Reem K. Arafa[2*], and Mohamed N. Seleem[1,5*]

[1] Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, 625 Harrison St., West Lafayette, IN 47907, USA

[2] Biomedical Sciences Program, University of Science and Technology, Zewail City of Science and Technology, Sheikh Zayed District, 6th of October City, Cairo, Egypt 12588

[3] Bindley Bioscience Center, Purdue University, 1201 W State St., West Lafayette, IN 47907, USA

[4] Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University, 575 Stadium Mall Dr., West Lafayette, IN 47907, USA

[5] Purdue Institute for Inflammation, Immunology, and Infectious Diseases, 610 Purdue Mall, West Lafayette, IN 47907, USA

*Corresponding Authors:

Reem K. Arafa
Biomedical Sciences Program
Zewail City of Science and Technology
Sheikh Zayed District
6th of October City
Cairo, Egypt 12588
Phone: +20 2 385 40 430 Fax: 20 2 385 17 181
Email: rkhidr@zewailcity.edu.eg

Mohamed N. Seleem
Department of Comparative Pathobiology
Purdue University College of Veterinary Medicine
625 Harrison St., West Lafayette, IN, 47907
Phone: 765-494-0763 Fax: 765-496-2627
Email: mseleem@purdue.edu

Supplementary Table 1: *S. cerevisiae* heterozygous diploid deletion strains identified as sensitive to compound 4b via chemogenomic profiling.

Gene Name	Gene Function[a]
ssa1	ATPase subunit of the chaperonin-containing T-complex (CCT, TriC)
nup60	FG-nucleoporin component of central core of the nuclear pore complex
kin3	Serine/threonine protein kinase
cox17	Copper metallochaperone that transfers copper to Sco1p and Cox11p
snf7	One of four subunits of the ESCRT-III complex; involved in the sorting of transmembrane proteins into the multivesicular body (MVB) pathway
ypl251w	Dubious open reading frame; unlikely to encode a functional protein
yah1	Ferredoxin of the mitochondrial matrix; required for formation of cellular iron-sulfur proteins; involved in heme A biosynthesis;
nsl1	Essential component of the MIND kinetochore complex; joins kinetochore subunits contacting DNA to those contacting microtubules; required for accurate chromosome segregation
aft2	Iron-regulated transcriptional activator; activates genes involved in intracellular iron use and required for iron homeostasis and resistance to oxidative stress
isu1	Conserved protein of the mitochondrial matrix; performs a scaffolding function during assembly of iron-sulfur clusters
grx3	Glutathione-dependent oxidoreductase
rli1	Essential Fe-S protein; required for ribosome biogenesis, translation initiation/termination
rim101	Cys2His2 zinc-finger transcriptional repressor; involved in alkaline responsive gene repression as part of adaptation to alkaline conditions; involved in cell wall assembly
sbe22	Protein involved in bud growth; involved in the transport of cell wall components from the Golgi to the cell surface
spc97	Component of the microtubule-nucleating Tub4p (gamma-tubulin) complex
nfs1	Cysteine desulfurase; involved in iron-sulfur cluster (Fe/S) biogenesis and in thio-modification of mitochondrial and cytoplasmic tRNAs
kdx1	Protein kinase; implicated in Slt2p mitogen-activated (MAP) kinase signaling pathway
isu2	Mitochondrial protein required for iron-sulfur protein synthesis
nej1	Protein involved in regulation of nonhomologous end joining
fks1	Catalytic subunit of 1,3-beta-D-glucan synthase; involved in cell wall synthesis and maintenance
htb1	Histone H2B; core histone protein required for chromatin assembly and chromosome function
mig2	Zinc finger transcriptional repressor
git1	Plasma membrane permease; mediates uptake of glycerophosphoinositol and glycerophosphocholine as sources of the nutrients inositol and phosphate

mrs4	Iron transporter of the mitochondrial carrier family; mediates Fe2+ transport across the inner mitochondrial membrane
sec28	Epsilon-COP subunit of the coatomer; regulates retrograde Golgi-to-ER protein traffic
fet4	Low-affinity Fe(II) transporter of the plasma membrane
sec31	Component of the Sec13p-Sec31p complex of the COPII vesicle coat; COPII coat is required for vesicle formation in ER to Golgi transport
sok1	Protein of unknown function
mrh1	Protein that localizes primarily to the plasma membrane; also found at the nuclear envelope
spc98	Component of the microtubule-nucleating Tub4p (gamma-tubulin) complex
gcd10	Subunit of tRNA (1-methyladenosine) methyltransferase with Gcd14p; required for the modification of the adenine at position 58 in tRNAs, especially tRNAi-Met
ecm33	GPI-anchored protein of unknown function; possible role in apical bud growth and hyphae formation (in *Candida*)
spt15	TATA-binding protein (TBP); general transcription factor that interacts with other factors to form the preinitiation complex at promoters
yjr039w	Putative protein of unknown function

[a]Information obtained from the *Saccharomyces* Genome Database

Supplementary Table 2: Minimum inhibitory concentration (MIC) of compound **4b**, in the presence of $CuSO_4$ or $FeSO_4$, screened against *S. cerevisiae* BY4743 and *C. albicans* P60002.

	MIC (µg/mL) for 4b	
Media supplemented with	***S. cerevisiae* BY4743**	***C. albicans* P60002**
50 µM $CuSO_4$	128	128
100 µM $CuSO_4$	>128	>128
50 µM $FeSO_4$	64	128
100 µM $FeSO_4$	128	128
500 µM $FeSO_4$	>128	128
1 mM $FeSO_4$	>128	>128

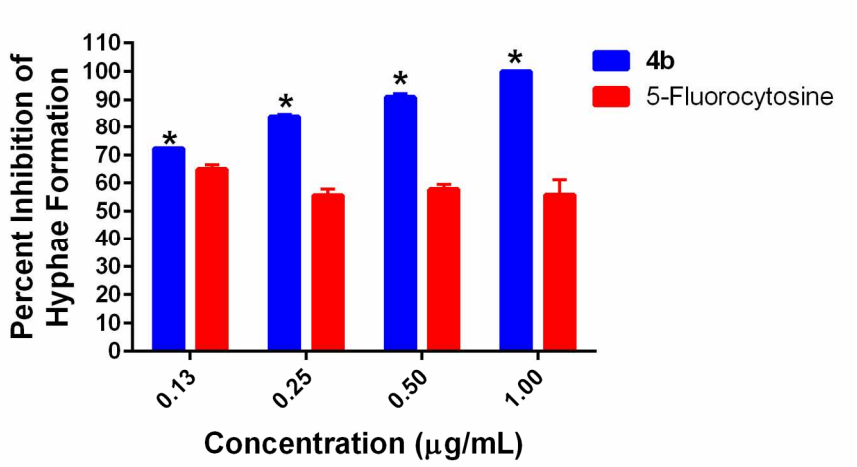
Supplementary Table 3: Aqueous turbidometric solubility assessment of compound **4b** and control drugs.

Compound/Drug	**Aqueous solubility limit[a] (µg/mL)**	**NOTES**
4b	>218	High solubility
Tamoxifen	6	Low solubility control
Verapamil	>227	High solubility control

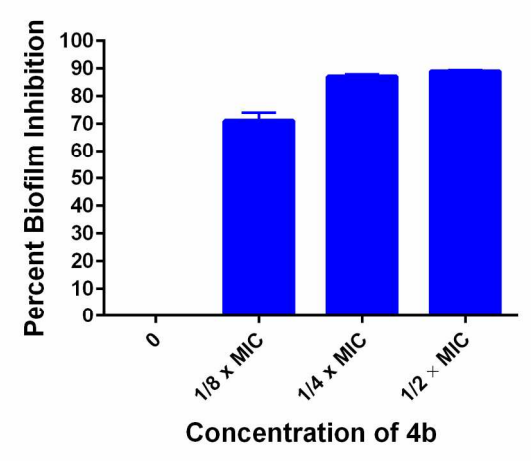
[a]Solubility limit corresponds to the highest concentration of test compound where no precipitate was detected (OD_{540})

Supplementary Table 4: Fungal strains used in this study.

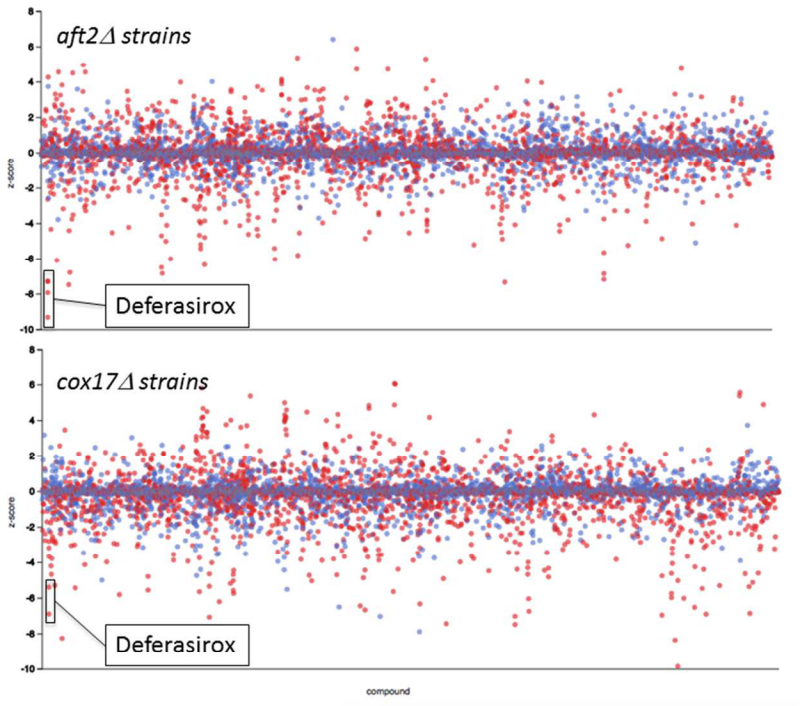
Strain Name	Alternative Strain Designation	Description
Candida albicans NR-29448	P60002	Isolated from a bloodstream infection, collected in Arizona, USA.
Candida albicans NR-29351	18M	Isolated from patient in China.
Candida albicans NR-29365	23F	Isolated from patient in China.
Candida albicans NR-29368	28C	Isolated from patient in China.
Candida albicans NR-29446	P94015	Isolated from a bloodstream infection collected in Utah, USA.
Candida albicans ATCC MYA-573	M4	Isolated from patient with AIDS in Germany. Resistant to fluconazole.
Candida albicans ATCC 64124	Darlington	Isolated from a mouth swab. Resistant to ketoconazole.
Candida krusei ATCC 14243		None.
Candida krusei ATCC 34135	ST-112	Clinical specimen isolated from Minnesota, USA.
Candida parapsilosis ATCC 22019	CBS 604	Isolated from a case of sprue in Puerto Rico
Candida glabrata ATCC MYA-2950	303542	None.
Candida glabrata ATCC 66032	AmMS 231	None.
Candida tropicalis ATCC 1369	CCY 29-7-7	None.
Candida tropicalis ATCC 13803	FDA PCl M-59	None.
Cryptococcus gattii NR-43208	R265	Isolated from a patient on Vancouver Island, Canada in late 1990s.
Cryptococcus gattii NR-43209	CBS1930	Isolated from a goat in Aruba prior to an outbreak in Vancouver, Canada.
Cryptococcus neoformans NR-41292	Isolate 2	Isolated from human cerebrospinal fluid in China in February 2012.
Aspergillus brasiliensis ATCC 16404	WLRI 034(120)	Isolated from North Carolina, USA.
Aspergillus niger ATCC 6275	4247	None.
Aspergillus niger ATCC 16888	WB 326	None.
Aspergillus fumigatus NR-35302	B5854	Isolated from human peritoneal fluid in California, USA in 1998.
Aspergillus fumigatus NR-35301	B5852	Isolated from human abdominal tissue in California, USA in 1998.



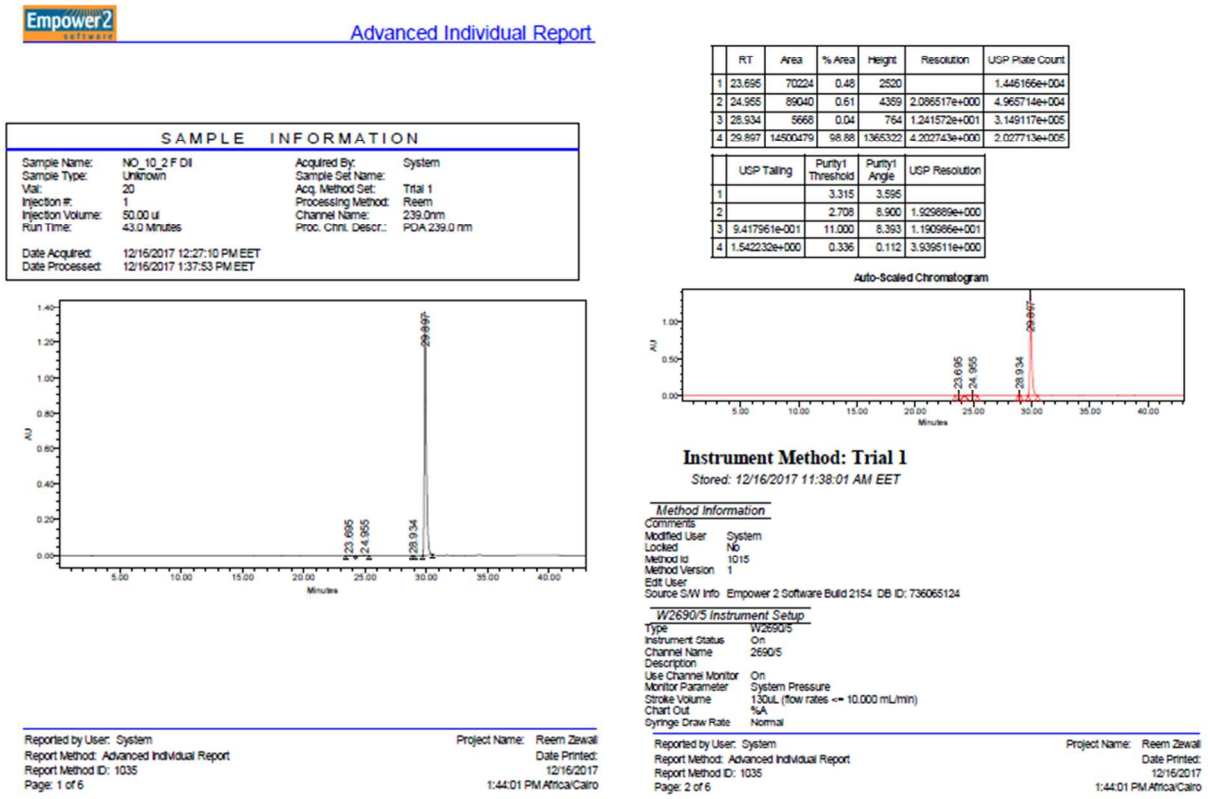
Supplementary Figure 1. Percent inhibition *C. albicans* P60002 hyphae formation. *C. albicans* (~6 × 10^5 CFU/mL) in RPMI-1640 medium supplemented with MOPS was exposed to **4b** (0.13 μg/ml up to 1 μg/mL), 5-Fluorocytosine (0.13 μg/ml up to 1 μg/mL), or left untreated for three hours at 35 ºC to induce yeast-to-hyphae transition. Morphological changes were observed via a Nikon TiS inverted microscope (40× objective lens) in at least two different fields of view and the number of yeast and filamentous cells were counted to determine the percent inhibition of hyphae formation. Data were analyzed via a two-way ANOVA with post-hoc Dunnet's test for multiple comparisons. Asterisks (*) indicate significant difference ($P < 0.05$) between values obtained for yeast treated with **4b** compared to 5-Fluorocytosine.



Supplementary Figure 2. Percent inhibition *C. albicans* P60002 biofilm formation. An overnight suspension of *C. albicans* was diluted in RPMI-1640 medium supplemented with MOPS (to achieve a starting inoculum ~5 x 10^5 CFU/mL) and exposed to **4b** (at subinhibitory concentrations) or left untreated for 24 hours at 37 ºC to permit biofilm formation. The biofilm was stained with 0.1% crystal violet, de-stained with ethanol, and biofilm mass quantified at OD_{595}. Data are presented as percent biofilm inhibition by **4b** in relation to the untreated control wells.



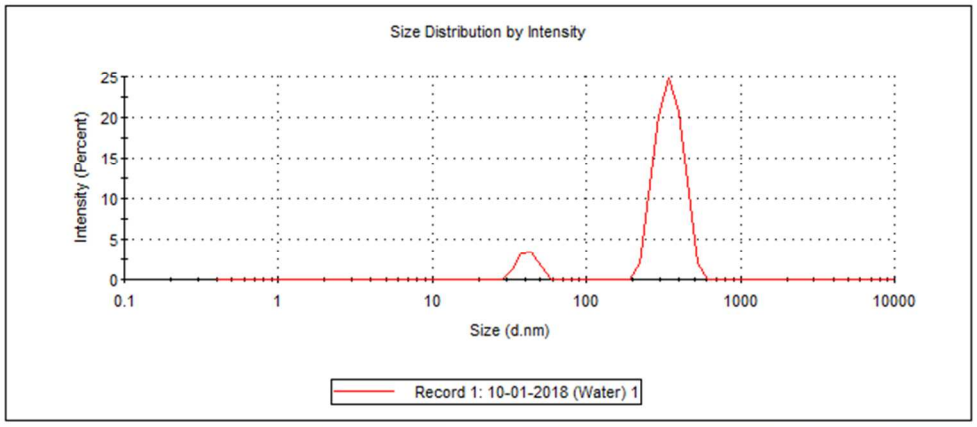
Supplementary Figure 3. Yeast chemogenomic profiling results for *aft2*Δ and *cox17*Δ deletion strains showing relative sensitivity of each strain against 1800 compounds. The graph depicts data for heterozygous (Red dots) and homozygous deletion strains (Blue dots) as plotted by z-score and compound. Negative z-score indicates increased sensitivity. The *aft2*Δ heterozygous strain (labeled black box) is most sensitive to deferasirox compared to 1800 compounds. The *cox17*Δ strain is also relatively sensitive to deferasirox.



Supplementary Figure 4. HPLC trace of compound **4b** appearing at 29.897 min.

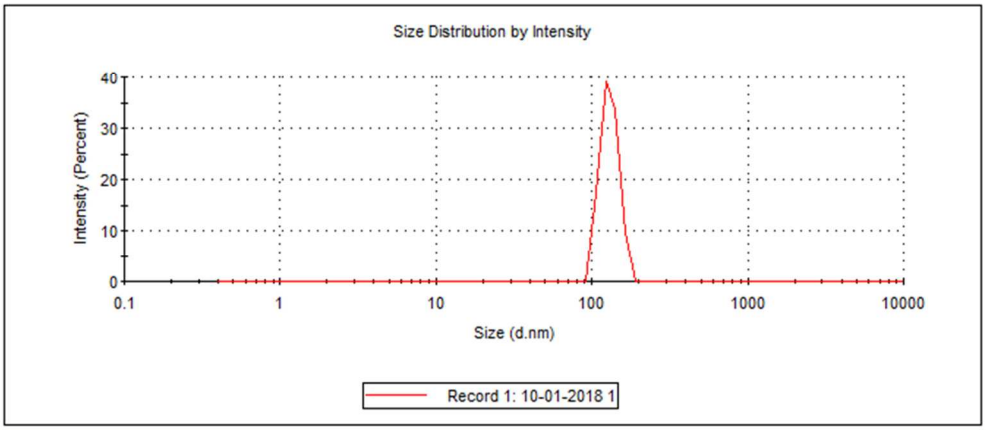
		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 485.3	Peak 1:	349.1	90.7	68.26
PdI: 0.506	Peak 2:	41.42	9.3	5.605
Intercept: 0.666	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 350.1	Peak 1:	130.0	100.0	16.86
PdI: 0.661	Peak 2:	0.000	0.0	0.000
Intercept: 0.601	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



Supplementary Figure 5. Dynamic Light Scattering result for compound **4b** in water (top panel) and DMSO (bottom panel).