Overcoming fluconazole resistance in *Candida albicans* clinical isolates with tetracyclic indoles

Willmen Youngsaye[a], Chris Dockendorff[a], Benjamin Vincent[b,c], Cathy L. Hartland[a], Joshua A. Bittker[a], Sivaraman Dandapani[a], Michelle Palmer[a], Luke Whitesell[b], Susan Lindquist[b,d], Stuart L. Schreiber[a,e], and Benito Munoz[a,*]

[a] *Chemical Biology Platform and Probe Development Center, Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142, USA*
[b] *Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA*
[c] *Microbiology Graduate Program, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139, USA*
[d] *Department of Biology and Howard Hughes Medical Institute, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139, USA*
[e] *Howard Hughes Medical Institute, Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, MA 02142, USA*

Supplemental Information

∗ Corresponding author. Tel.: +1 617 714 7225; fax: +1 617 714 8969

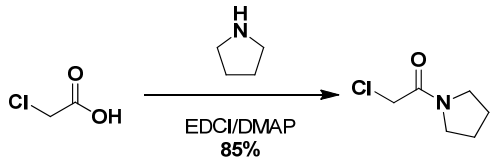
E-mail address: bmunoz@broadinstitute.org (B. Munoz)

Chemistry Experimental Methods

General details. All reagents and solvents were purchased from commercial vendors and used as received. NMR spectra were recorded on a Bruker 300 MHz or Varian 500 MHz spectrometer using $CDCl_3$, or DMSO-d_6 solvents, as indicated. Proton and carbon chemical shifts are reported in ppm (δ) relative to tetramethylsilane (^{1}H δ 0.00) or residual chloroform in $CDCl_3$ solvent (^{1}H δ 7.24, ^{13}C δ 77.0). NMR data are reported as follows: chemical shifts, multiplicity (obs. = obscured, br = broad, s = singlet, d = doublet, t = triplet, q = quartet, pent = pentet, m = multiplet); coupling constant(s) in Hz; integration.

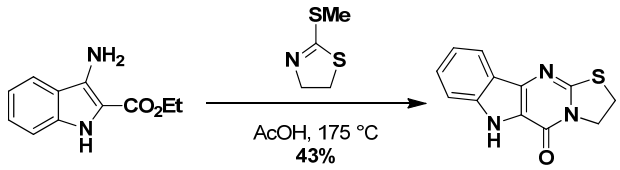
Unless otherwise indicated, NMR data were collected at 25 °C. Flash chromatography was performed using 40-60 μm Silica Gel (60 Å mesh) on a Teledyne Isco Combiflash R_f system. Tandem Liquid Chromatography/Mass Spectrometry (LC/MS) was performed on a Waters 2795 separations module and 3100 mass detector. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F plates. Visualization was accomplished with UV light and aqueous potassium permanganate ($KMnO_4$) stain followed by heating. High-resolution mass spectra were obtained at the MIT Mass Spectrometry Facility with a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance mass spectrometer. X-ray crystallography was performed at the MIT Department of Chemistry X-Diffraction Facility with a Siemens three-circle Platform diffractometer, coupled to a Bruker-APEX CCD detector.

Compound purity and identity were determined by UPLC-MS (Waters, Milford, MA). Purity was measured by UV absorbance at 210 nm. Identity was determined on an SQ mass spectrometer by positive electrospray ionization. Mobile Phase A consisted of either 0.1% ammonium hydroxide or 0.1% trifluoroacetic acid in water, while mobile Phase B consisted of the same additives in acetonitrile. The gradient ran from 5% to 95% mobile Phase B over 0.8 minutes at 0.45 ml/min. An Acquity BEH C18, 1.7 μm, 1.0 x 50 mm column was used with column temperature maintained at 65°C. Compounds were dissolved in DMSO at a nominal concentration of 1 mg/ml, and 0.25 μl of this solution was injected.

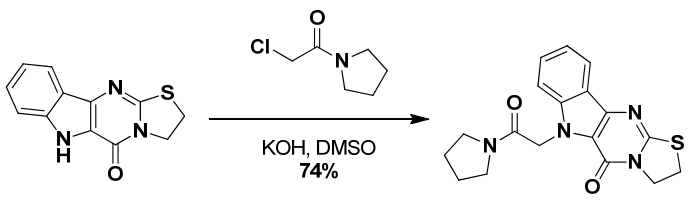


2-Chloro-1-(pyrrolidin-1-yl)ethanone: A round-bottom flask equipped with a magnetic stir bar was charged with chloroacetic acid (1.3 mL, 21.7 mmol, 3.0 equiv) and dichloromethane (17.6 mL). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.7 g, 14.1 mmol, 2.0 equiv) and 4-dimethylaminopyridine (86 mg, 0.70 mmol, 0.1 equiv) were added to the clear, colorless solution. Neat pyrrolidine (0.6 mL, 7.2 mmol, 1.0 equiv) was added to the

reaction, and the resulting clear yellow solution was stirred at room temperature for 22 hours. The reaction was quenched with 1.0 M hydrochloric acid solution (aqueous, 10 mL) and stirred for 5 minutes at room temperature to give a clear, yellow solution. The layers were separated, and the aqueous phase was extracted with dichloromethane (3 x 10 mL). The combined organic layers were washed with 1.0 M hydrochloric acid solution (aqueous, 25 mL), saturated sodium bicarbonate solution (aqueous, 2 x 25 mL), and lastly with brine (25 mL). The organic phase was shaken over magnesium sulfate, filtered, and concentrated under reduced pressure to give red-brown oil. The crude material was purified by column chromatography over silica gel (hexanes/ethyl acetate: 100/0 to 0/100) to give the title compound as an ivory-colored flaky solid (0.90 g, 85% yield). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 4.03 (s, 2H), 3.52 (dd, *J* = 6.6, 11.5 Hz, 4H), 1.98 (d pent, *J* = 6.5, 20.3 Hz, 4H); **MS (ESI$^+$):** $C_6H_{11}ClNO$ [M+H] found 148.18, calculated 148.05.



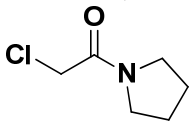
2,3-Dihydrothiazolo[3',2':1,2]pyrimido[5,4-*b*]indol-5(6*H*)-one (6a): Ethyl 3-amino-1H-indole-2-carboxylate (300 mg, 1.5 mmol, 1.0 equiv) was placed in a sealed tube equipped with a magnetic stir bar. Glacial acetic acid (2.9 mL) was added, followed by 2-(methylthio)-4,5-dihydrothiazole (170 µL, 1.6 mmol, 1.1 equiv). The resulting red-orange suspension was sealed under nitrogen and heated to 175 °C, where it was stirred for 2 hours to give a clear, dark red solution. The reaction was cooled to room temperature to give a thick, tan suspension. Saturated sodium bicarbonate solution (aqueous, 75 mL) was carefully added to the reaction and resulting mixture was further diluted with dichloromethane (30 mL). The layers were separated and the aqueous phase was extracted with ethyl acetate (4 x 50 mL). The combined organics were washed with brine (50 mL) then shaken over magnesium sulfate, filtered, and concentrated under reduced pressure to give a golden brown solid. The crude material was purified by column chromatography over silica gel (hexanes/ethyl acetate: 100/0 to 0/100) to give the desired product as a pale yellow solid (0.15 g, 43 %). **^{1}H NMR (300 MHz, DMSO)** δ 12.03 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.52 – 7.38 (m, 2H), 7.19 (ddd, *J* = 8.0, 6.7, 1.3 Hz, 1H), 4.50 (t, *J* = 7.4 Hz, 2H), 3.62 (t, *J* = 7.4 Hz, 2H); **MS (ESI$^+$):** $C_{12}H_{10}N_3OS$ [M+H] found 244.19 calculated 244.05.

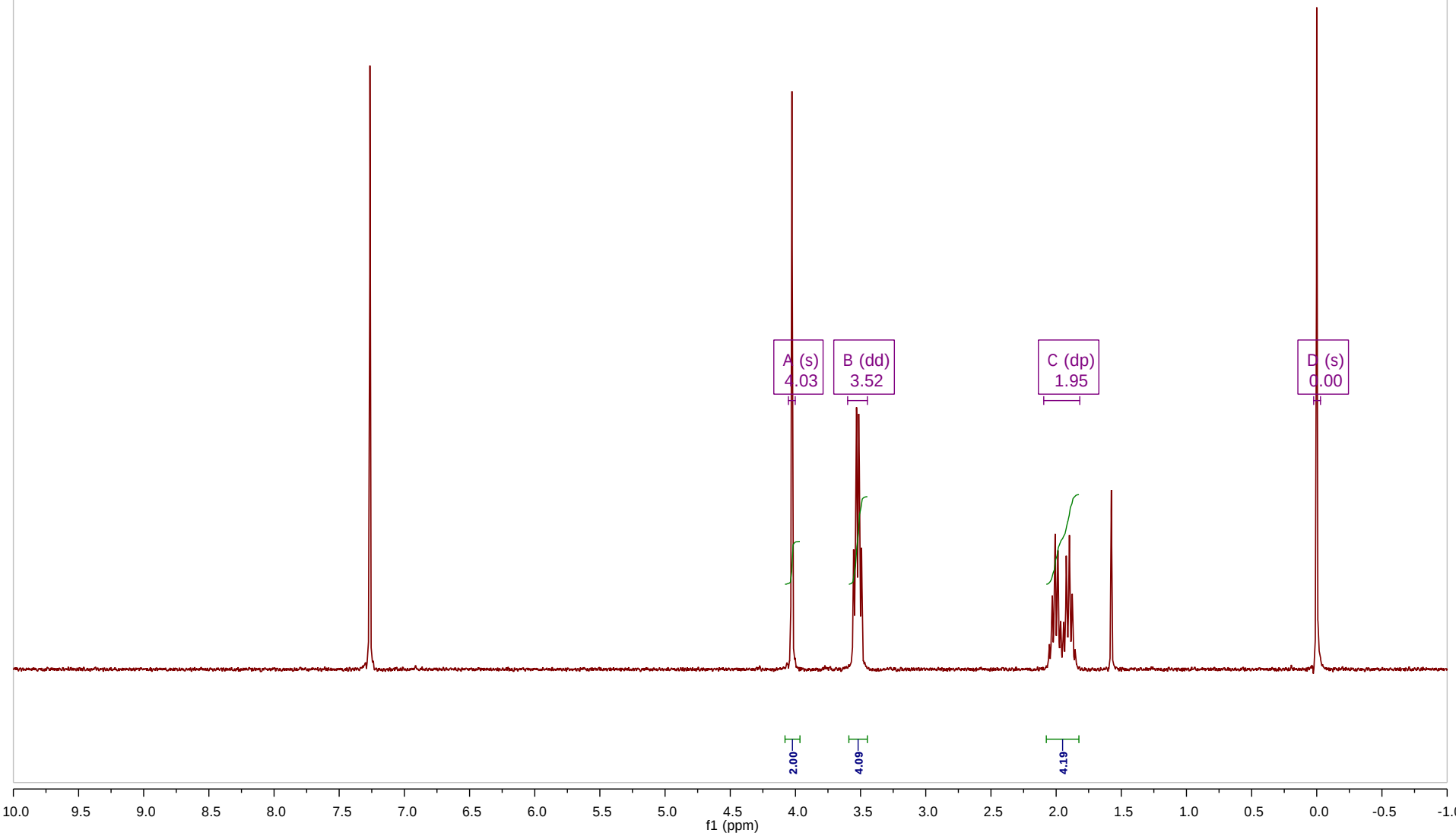


6-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-2,3-dihydrothiazolo-[3',2':1,2]-pyrimido-[5,4-*b*]-indol-5(6*H*)-one: 2,3-dihydrothiazolo-[3',2':1,2]-pyrimido-[5,4-*b*]-indol-5(6*H*)-one **6** (31 mg, 0.13 mmol, 1.0 equiv) and powdered potassium hydroxide (11 mg, 0.19 mmol, 1.5 equiv) were added to a vial equipped with a magnetic stir bar. The vial was sealed

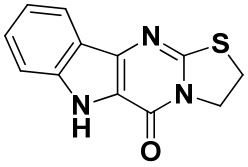
with a septum cap and flushed with nitrogen, before anhydrous dimethyl sulfoxide (600 μL) was added. The mixture was stirred for 30 minutes at room temperature, then a solution of 2-chloro-1-(pyrrolidin-1-yl)ethanone (30 mg, 0.20 mmol, 1.5 equiv) in dimethyl sulfoxide (0.3 mL) was added via syringe. An additional 0.3 mL of dimethyl sulfoxide was used to ensure complete transfer of the amide. The reaction was stirred at room temperature for 14 hours, generating a fine, white precipitate. Water (0.5 ml) was added to the vial, and the reaction was thoroughly mixed. The fine precipitate was filtered out through a cotton plug with additional water (1 mL). The collected solid was then dissolved with 1:1 (v/v) methanol/dichloromethane (approximately 3 mL) and adsorbed on silica gel before being purified by column chromatography over silica gel (methanol/dichloromethane: 0/100 to 10/90) to give the title compound as a white solid (33 mg, 74% yield). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 8.10 (d, *J* = 8.0, 1H), 7.48 (t, *J* = 7.7, 1H), 7.36 (d, *J* = 8.4, 1H), 7.25 (t, *J* = 5.0, 1H), 5.42 (s, 2H), 4.54 (t, *J* = 7.4, 2H), 3.59 (t, *J* = 6.8, 2H), 3.52 (t, *J* = 6.3, 2H), 3.49 (t, *J* = 6.3, 2H), 2.08 (apparent dt, *J* = 6.8, 6.8, 2H), 1.92 (apparent dt , *J* = 6.9, 6.9 ,2H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 165.8, 155.4, 155.0, 141.0, 140.5, 127.9, 121.2, 120.8, 120.5, 119.0, 110.0, 48.2, 46.7, 46.2, 45.7, 27.4, 26.3, 24.0; **HRMS (ESI):** calculated mass for $C_{18}H_{18}N_4O_2S$ [M+H] 355.1223, found 355.1231.

^{1}H NMR (300 MHz, $CDCl_3$)

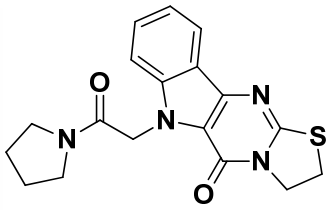




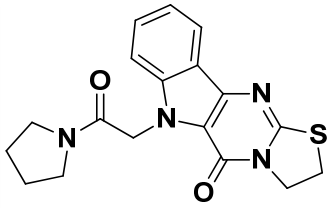
^{1}H NMR (300 MHz, DMSO-d_6)



^{1}H NMR (500 MHz, $CDCl_3$)



^{1}H NMR (125 MHz, $CDCl_3$)



	ppm	Intensity	Width	Type
1	165.76	857.4	1.31	Compound
2	155.37	425.7	1.34	Compound
3	154.99	576.9	1.27	Compound
4	140.98	712.8	1.30	Compound
5	140.50	499.8	1.28	Compound
6	127.94	1653.8	1.61	Compound
7	121.21	1767.4	1.60	Compound
8	120.82	1718.0	1.60	Compound
9	120.49	699.0	1.24	Compound
10	119.04	461.6	1.36	Compound
11	110.03	1733.5	1.61	Compound
12	77.25	6531.8	2.71	Solvent
13	77.00	6697.4	2.68	Solvent
14	76.75	6625.6	2.72	Solvent
15	48.23	1756.4	1.77	Compound
16	46.66	1681.8	1.92	Compound
17	46.15	1792.7	1.72	Compound
18	45.69	1759.5	1.73	Compound
19	27.40	1842.6	1.63	Compound
20	26.26	1993.8	1.50	Compound
21	24.00	1885.6	1.48	Compound