Supporting Information

Decoupling Activation of Heme Biosynthesis from Anaerobic Toxicity in a Molecule Active in *Staphylococcus aureus*

Brendan F. Dutter[1], Laura A. Mike[2], Paul R. Reid[1], Katherine M. Chong[3], Susan Ramos-Hunter[3], Eric P. Skaar[1,2], Gary A. Sulikowski[1,3]

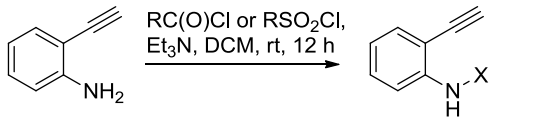
[1]Vanderbilt Institute of Chemical Biology; [2]Department of Pathology, Microbiology and Immunology; Vanderbilt University Medical Center, Nashville, TN 37232
[3]Department of Chemistry, Vanderbilt University, Nashville, TN 37232

1. General Procedure: All non-aqueous reactions were performed in flame-dried flasks under an atmosphere of argon. Stainless steel syringes were used to transfer air- and moisture-sensitive liquids. Reaction temperatures were controlled using a thermocouple thermometer and analog hotplate stirrer. Reactions were conducted at room temperature (rt, approximately 23 °C) unless otherwise noted. Flash column chromatography was conducted using silica gel 230-400 mesh. Analytical thin-layer chromatography (TLC) was performed on E. Merck silica gel 60 F254 plates and visualized using UV and iodine stain.

2. Materials: All solvents and chemicals were purchased from Sigma-Aldrich unless otherwise noted. Dry dichloromethane was collected from an MBraun MB-SPS solvent system. N,N-dimethylformamide (DMF), tetrahydrofuran (THF), and acetonitrile (MeCN) were used as received in a bottle with a Sure/Seal. Triethylamine was distilled from calcium hydride and stored over KOH. Deuterated solvents were purchased from Cambridge Isotope Laboratories.

3. Instrumentation: ^{1}H NMR spectra were recorded on Bruker 400, 500, or 600 MHz spectrometers and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad, app = apparent), coupling constants (Hz), and integration. ^{13}C NMR spectra were recorded on Bruker 100, 125, or 150 MHz spectrometers and are reported relative to deuterated solvent signals. Low resolution mass spectrometry (LRMS) was conducted and recorded on an Agilent Technologies 6130 Quadrupole instrument.

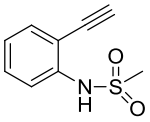
4. Synthetic procedures and compound characterization data:



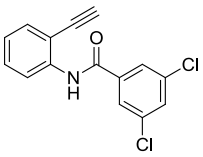
X = SO_2Me, or C(O)R

2-ethynyl-N-acylbenzamides: To a stirred solution of 2-ethynylaniline (1.0 eq) dissolved in dichloromethane (0.3 M) at room temperature was added acyl or sulfonyl chloride (1.0 eq).

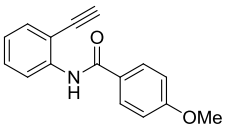
Tiriethylamine was slowly added to the reaction and once addition was complete, the reaction was stirred at room temperature overnight. Solvents were removed *in vacuo* and the residue was partitioned between ethyl acetate and saturated $NaHCO_3$ (aq), the organic layer dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography.



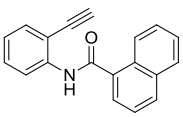
N-(2-ethynylphenyl)methanesulfonamide (S1). Light brown solid; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J=8.20 Hz, 1H) 7.50 (dd, J=7.72 Hz, J=1.40 Hz, 1H), 7.39 (br t, J=7.90 Hz, 1H), 7.13 (t, J=7.67 Hz, 1H), 7.02 (br, 1H), 3.49 (s, 1H), 3.02 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 139.6, 133.0, 130.7, 124.8, 119.6, 113.0, 84.9, 78.8, 39.8; LRMS calculated for $C_9H_9NO_2S$ $(M+H)^+$ *m/z*: 196.0, measured 196.1.



N-(2-ethynylphenyl)-4-methoxybenzamide (S2). Off-white solid; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.64 (br, 1H), 8.51 (d, J=8.28 Hz, 1H), 7.77 (d, J=1.84 Hz, 2H), 7.57 (t, J=1.86 Hz, 1H), 7.44 (t, J=7.95 Hz, 1H), 7.12 (td, J=7.57 Hz, 1.05 Hz, 1H), 3.64 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 162.7, 139.2, 137.8, 136.0, 132.4, 132.1, 130.6, 125.9, 124.3, 119.6, 111.5, 85.3, 79.4; LRMS calculated for $C_{15}H_9Cl_2NO$ $(M+H)^+$ *m/z*: 290.0, measured 290.0.

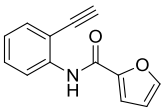


3,5-dichloro-N-(2-ethynylphenyl)benzamide (S3). White solid; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.72 (br, 1H), 8.59 (d, J=8.20 Hz, 1H), 7.89 (d, J=6.78 Hz, 2H), 7.50 (dd, J=7.68 Hz, 1.48 Hz, 1H), 7.42 (t, J=7.95 Hz, 1H), 7.06 (td, J=7.54 Hz, 1.08 Hz, 1H), 7.00 (d, J=8.84 Hz, 2H), 3.88 (s, 3H), 3.59 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 164.9, 162.8, 140.2, 132.3, 130.5, 129.1, 127.2, 123.3, 119.3, 114.3, 110.9, 84.7, 79.7, 55.6; LRMS calculated for $C_{16}H_{13}NO_2$ $(M+H)^+$ *m/z*: 252.1, measured 252.1.

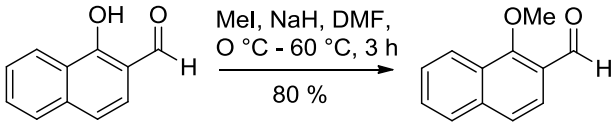


N-(2-ethynylphenyl)-1-naphthamide (S4). White solid; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J=8.20 Hz, 1H), 8.57 (br s, 1H), 8.50 (d, J=8.24 Hz, 1H), 8.00 (d, J=8.00 Hz, 1H), 7.92 (d, J=7.64 Hz, 1H), 7.83 (d, J=7.04 Hz, 1H), 7.63-7.45 (m, 5H), 7.12 (t, J=7.52 Hz, 1H), 3.42 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 167.5, 140.1, 134.3, 134.0, 132.4,

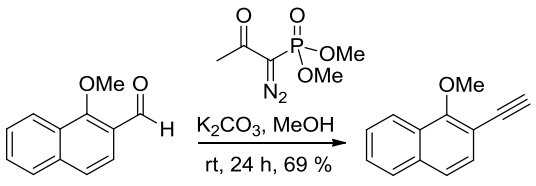
131.6, 130.5, 130.3, 128.6, 127.6, 126.8, 125.6, 125.5, 124.9, 123.8, 119.7, 111.3, 84.9, 79.3 ; LRMS calculated for $C_{19}H_{13}NO$ $(M+H)^+$ *m/z*: 272.1, measured 272.1.



N-(2-ethynylphenyl)furan-2-carboxamide (S5). Brown solid; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.99 (br, 1H), 8.55 (d, J=8.24 Hz, 1H), 7.53 (s, 1H), 7.49 (dd, J=7.68 Hz, 1.48 Hz, 1H), 7.40 (t, J=7.94 Hz, 1H), 7.25 (d, J=3.54 Hz, 1H), 7.06 (t, J=7.71 Hz, 1H), 6.56 (dd, J=3.50 Hz, 1.72 Hz, 1H), 3.60 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.1, 148.0, 144.7, 139.4, 132.3, 130.4, 123.6, 119.4, 115.6, 112.7, 111.1, 84.8, 79.1 ; LRMS calculated for $C_{13}H_9NO_2$ $(M+H)^+$ *m/z*: 212.1, measured 212.1.



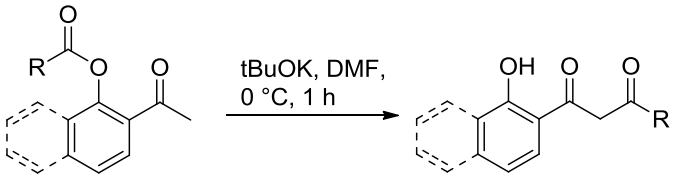
1-methoxy-2-naphthaldehyde (S6). To a stirred solution of 193 mg (1.12 mmol, 1.0 eq) 1-hydroxy-2-naphthaldehyde (TCI America) dissolved in 5 mL N,N-dimethylformamide at 0 °C was added 49.0 mg (1.23 mmol, 1.2 eq) sodium hydride. The mixture was stirred at 0 °C for 5 min and 140 µL (2.24 mmol, 2.0 eq) methyl iodide was added. The reaction was heated to 60 °C and stirred for 3 h. The reaction was partitioned between ethyl acetate and water, the organic layer washed with water (1x), brine (2x), and dried ($MgSO_4$). The organic layer was concentrated and the residue purified by flash chromatography with a 0-20 % ethyl acetate in hexane gradient to provide 165 mg (80 %) of product as a light brown solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.59 (d, J=0.76 Hz, 1H), 8.23 (d, J=8.16 Hz, 1H), 7.86 – 7.82 (m, 2H), 7.64 - 7.54 (m, 3H), 4.12 (s, 3H); ^{13}C-NMR (100 MHz) δ 189.6, 162.6, 138.1, 129.4, 128.4, 127.9, 126.9, 124.9, 124.7, 123.2, 122.7, 65.7; LRMS calculated for $C_{12}H_{10}O_2$ $(M+H)^+$ *m/z*: 187.1, measured 187.1.



2-ethynyl-1-methoxynaphthalene (S7). To a stirred solution of 106 mg (0.570 mmol, 1.0 eq) 1-methoxy-2-naphthaldehyde in 5 mL methanol was added 158 mg (1.14 mmol, 2.0 eq) potassium carbonate followed by 131 mg (0.682 mmol, 1.2 eq) dimethyl (1-diazo-2-oxopropyl)phosphonate. The suspension was stirred for 24 h. The reaction was partitioned between ethyl acetate and water, the aqueous layer extracted with ethyl acetate (2x), the organics combined and washed with brine (1x), dried ($MgSO_4$), and flash filtered through silica with 5:1 hexane/ethyl acetate to provide 72 mg (69 %) 2-ethynyl-1-methoxynaphthaldehyde. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.97 (m, 1H), 7.62 – 7.58 (m, 1H), 7.35 – 7.27 (m, 4H), 3.97 (s, 3H), 3.22 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 159.3, 144.2, 134.9, 129.8, 127.9, 127.4, 126.5, 123.5, 122.5, 110.3, 82.5, 80.8, 61.9; LRMS calculated for $C_{13}H_{10}O$ $(M+H)^+$ *m/z*: 183.1, measured 183.1.

The alkyne precursor for **3a** and **3b** was prepared from *o*-anisaldehyde as previously described.[1]

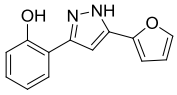
2'-acyloxyacetophenone synthesis. To a stirred solution of 2'-hydroxyacetophenone in dichloromethane (0.2 M) was added triethylamine (1.1 eq) and 4-dimethylaminopyridine (0.05 eq). The solution was cooled to 0 °C in an ice bath and acid chloride (1.1 eq) was added. The reaction was allowed to warm to room temperature and monitored by TLC and LCMS. When starting material was completely consumed and product observed by LC-MS, the reaction was partitioned between dichloromethane and saturated $NaHCO_3$ (aq). The organic layer was washed with brine and dried ($MgSO_4$), filtered, and concentrated. The products generally did not require further purification but may be crystallized from hexane/ethyl acetate or purified by flash chromatography as needed. Products were generally carried on uncharacterized.



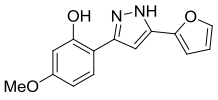
1,3-diketone synthesis. A suspension of potassium *tert*-butoxide (2.0 eq) in dimethylformamide (0.2 M) was cooled to 0 °C under Ar. A solution of 2'-acyloxyacetophenone (1.0 eq) in DMF was added dropwise to the *t*BuOK suspension and stirred at 0 °C until consumption of starting material was observed by TLC (~1 h). The reaction was quenched with 1 N HCl and the resulting suspension extracted with diethyl ether (3x), washed with H_2O, brine, dried ($MgSO_4$) and solvents removed *in vacuo*. Products were carried on crude and uncharacterized.

General procedure for demethylation of aryl ethers. To a solution of reactant in dichloromethane (0.20 M) was added 6.0 eq BBr_3 (1.0 M in dichloromethane) in a microwave vial. The vial was sealed and maintained at 90 °C under microwave irradiation for 20 min. The reaction was quenched with saturated $NaHCO_3$ and extracted with dichloromethane. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. Products were purified by flash chromatography or HPLC.

1 and **2e** were previously reported.[2]

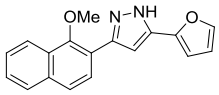


2-(5-(furan-2-yl)-1H-pyrazol-3-yl)phenol (1a) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.76 (d, J=7.78 Hz, 1H), 7.72 (s, 1H), 7.22 (t, J=7.73 Hz, 1H), 7.12 (s, 1H), 6.96-6.90 (m, 3H), 6.63 (dd, J=3.39 Hz, 1.86 Hz, 1H); ^{13}C-NMR (150 MHz, acetone-d6) δ 158.3, 154.6, 144.1, 130.0, 127.6, 120.1, 117.6, 117.5, 112.7, 108.5, 99.2; LRMS calculated for $C_{13}H_{11}N_2O_2$ $(M+H)^+$ *m/z*: 227.1, measure, 227.1.

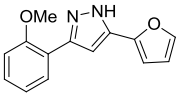


2-(5-(furan-2-yl)-1H-pyrazol-3-yl)-5-methoxyphenol (1b) ^{1}H-NMR (400 MHz, acetone-d6) δ 12.75 (br, 1H), 10.95 (br; 1H), 7.70 (s, 1H), 7.65 (d,

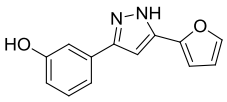
J=8.44 Hz, 1H), 7.00 (s, 1H), 6.91 (s, 1H), 6.62 (br, 1H), 6.54-6.48 (m, 2H), 3.80 (s, 3H); ^{13}C-NMR (100 MHz, acetone-d6) δ 161.9, 158.4, 144.0, 128.5, 112.6, 110.7, 108.3, 106.8, 102.4, 98.5, 55.4; .LRMS calculated for $C_{14}H_{12}N_2O_3$ $(M+H)^+$ *m/z*: 257.1, measured 257.1.



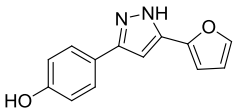
5-(furan-2-yl)-3-(1-methoxynaphthalen-2-yl)-1H-pyrazole (1c) ^{1}H-NMR (400 MHz, $CDCl_3$) δ]8.18 (d, J=8.20 Hz, 1H), 7.86 (d, J=7.80 Hz, 1H), 7.76 (d, J=8.60 Hz, 1H), 7.70 (d, J=8.64 Hz, 1H), 7.61 – 7.50 (m, 2H), 6.99 (s, 1H), 6.81 (d, J=3.28 Hz, 1H), 6.52 (dd, J=3.28 Hz, J=1.80, 1H), 3.92 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 152.8,148.8, 142.0, 134.9, 128.3, 128.2, 127.0, 126.9, 125.3, 122.4, 117.7, 111.5, 106.0, 101.1, 62.0; LRMS calculated for $C_{18}H_{14}N_2O_2$ $(M+H)^+$ *m/z*: 291.1, measured 291.1.



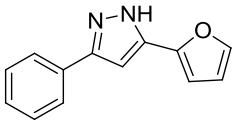
5-(furan-2-yl)-3-(2-methoxyphenyl)-1H-pyrazole (1d) ^{1}H-NMR (400 MHz, CD_3OD) δ 7.73 (d, J=7.20 Hz, 1H), 7.57 (s, 1H), 7.38 (t, J=6.8 Hz, 1H), 7.17 (d, J=8.4 Hz, 1H), 7.06 (t, J=7.60 Hz, 1H), 6.94 (s, 1H), 6.76 (d, J=3.20 Hz, 1H), 6.54 (m, 1H), 3.99 (s, 3H); ^{13}C-NMR (100 MHz, acetone-d6) δ 157.1, 150.3, 142.7, 142.5, 130.2, 128.7, 121.8, 119.3, 112.6, 112.1, 105.9, 101.5, 56.0; LRMS calculated for $C_{14}H_{12}N_2O_2$ $(M+H)^+$ *m/z*: 241.1, measured 241.1.



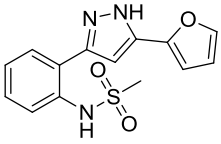
3-(5-(furan-2-yl)-1H-pyrazol-3-yl)phenol (1e) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.62 (s, 1H), 7.36 - 7.31 (m, 2H), 7.26 (t, J=7.77 Hz, 1H), 6.89 (s, 1H), 6.83 (d, J=8.18 Hz, 1H), 6.77 (d, J=3.19 Hz, 1H), 6.56 (dd, J=3.35 Hz, 1.41 Hz, 1H); ^{13}C-NMR (100 MHz, acetone-d6) δ 158.7, 143.0, 130.7, 117.6, 115.9, 113.2, 112.3, 106.6, 99.9; LRMS calculated for $C_{13}H_{10}N_2O_2$ $(M+H)^+$ *m/z*: 227.1, measured 227.2.



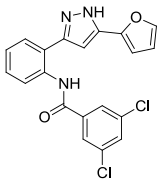
3-(5-(furan-2-yl)-1H-pyrazol-3-yl)phenol (1f) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.70 (d, J=8.57 Hz, 2H), 7.61 (d, J=1.24 Hz, 1H), 6.92 (d, J=8.57 Hz, 2H), 6.81 (s, 1H), 6.75 (d, J=3.24 Hz, 1H), 6.54 (dd, J=3.24 Hz, 1.24 Hz, 1H); ^{13}C-NMR (150 MHz, acetone-d6) δ 158.4, 157.3, 149.1, 148.0, 142.8, 127.7, 123.8, 116.5, 112.2, 106.2, 99.0; LRMS calculated for $C_{13}H_{10}N_2O_2$ $(M+H)^+$ *m/z*: 227.1, measured 227.1.



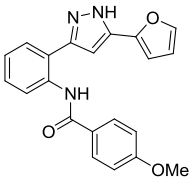
5-(furan-2-yl)-3-phenyl-1H-pyrazole (1g) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.77 (d, J=7.57 Hz, 2H), 7.57 (s, 1H), 7.44 (t, J=7.32 Hz, 2H), 7.35 (t, J=7.28 Hz, 1H), 6.87 (s, 1H), 8.77 (d, J=3.28 Hz, 1H), 6.54 (dd, J=3.28 Hz, 1.48 Hz, 1H); ^{13}C-NMR (150 MHz, acetone-d6) δ 143.1, 129.7, 128.8, 126.2, 112.3, 106.7, 9.8; LRMS calculated for $C_{13}H_{10}N_2O$ $(M+H)^+$ *m/z*: 211.1, measured 211.1.



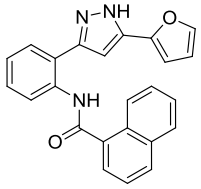
N-(2-(5-(furan-2-yl)-1H-pyrazol-3-yl)phenyl)methanesulfonamide (1h) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.91 (d, J=7.94 Hz, 1H), 7.74 (s, 1H), 7.71 (d, J=7.72 Hz, 1H), 7.22 (t, J=7.60 Hz, 1H), 7.16 (s, 1H), 6.95 (d, J=3.40 Hz, 1H), 6.64 (dd, J=3.40 Hz, 1.80 Hz, 1H), 2.97-2.95 (m, 3H); ^{13}C-NMR (100 MHz, acetone-d6) δ 152.6, 145.4, 144.2, 137.0, 136.5, 129.6, 129.1, 124.5, 121.5, 120.3, 112.7, 108.5, 100.7, 39.6; LRMS calculated for $C_{14}H_{13}N_3O_3S$ $(M+H)^+$ *m/z*: 304.1, measured 304.0.



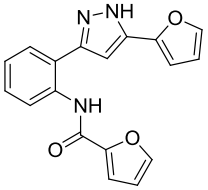
3,5-dichloro-N-(2-(5-(furan-2-yl)-1H-pyrazol-3-yl)phenyl)benzamide (1i) ^{1}H-NMR (400 MHz, acetone-d6) δ 8.82 (d, J=8.20 Hz, 1H), 8.01 (d, J=1.92 Hz, 2H), 7.93 (dd, J=7.82 Hz, 1.46 Hz, 1H), 7.72 (br s, 2H), 7.39 (br t, J=7.84 Hz, 1H), 7.23 (br t, J=7.58 Hz, 1H), 7.19 (s, 1H), 6.94 (d, J=3.28 Hz, 1H), 6.63 (dd, J=3.40 Hz, 1.84 Hz, 1H); ^{13}C-NMR (125 MHz, acetone-d6) 163.2, 145.4, 144.2, 140.0, 137.4, 136.2, 132.0, 129.4, 128.9, 127.0, 124.7, 121.4, 121.3. 112.7, 108.7, 101.2; LRMS calculated for $C_{20}H_{13}Cl_2N_3O_2$ $(M+H)^+$ *m/z*: 398.1, measured 398.0.



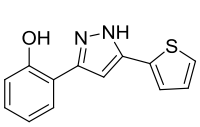
N-(2-(5-(furan-2-yl)-1H-pyrazol-3-yl)phenyl)-4-methoxybenzamide (1j) ^{1}H-NMR (400 MHz, acetone-d6) δ 8.95 (d, J=7.84 Hz, 1H), 8.16 (d, J=8.88 Hz, 2H), 7.90 (d, J=7.84 Hz, 1H), 7.71 (s, 1H), 7.36 (t, J=7.89 Hz, 1H), 7.19 - 7.14 (m, 2H), 7.05 (d, J=8.89 Hz, 2H), 6.94 (d, J=3.36 Hz, 1H), 6.62 (dd, J=3.38 Hz, 1.82 Hz, 1H), 3.88 (s, 3H); ^{13}C-NMR (100 MHz, acetone-d6) δ 165.5, 163.4, 144.1, 138.3, 130.2, 129.3, 128.7, 123.6, 121.1, 114.7, 112.7, 108.5, 101.0, 55.9; LRMS calculated for $C_{21}H_{17}N_3O_3$ $(M+H)^+$ *m/z*: 360.1, measured 360.1.



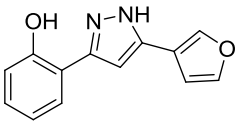
N-(2-(5-(furan-2-yl)-1H-pyrazol-3-yl)phenyl)-1-naphthamide (1k) ^{1}H-NMR (600 MHz, acetone-d6) δ 9.00 (d, J=8.28 Hz, 1H), 8.58 - 8.53 (m, 1H), 8.10 (d, J=8.24 Hz, 1H), 8.02 - 7.99 (m, 1H), 7.97 (d, J=7.97 Hz, 1H), 7.92 (dd, J=7.84 Hz, 1.48 Hz, 1H), 7.68 (d, J=1.44 Hz, 1H), 7.64 - 7.56 (m, 2H), 7.44 (td, J=7.88 Hz, 1.48 Hz, 1H), 7.24 (td, J=7.63 Hz, 0.96 Hz, 1H), 7.14 (s, 1H), 6.90 (d, J=3.36 Hz, 1H), 6.60 (dd, J=3.40 Hz, 1.80 Hz, 1H); ^{13}C-NMR (150 MHz, acetone-d6) δ 168.1, 153.0, 145.5, 144.1, 142.3, 138.0, 136.3, 134.9, 131.3, 131.4, 129.3, 129.2, 128.8, 127.7, 127.3, 126.6, 126.4, 126.0, 124.3, 121.3, 112.6, 108.3, 101.1; LRMS calculated for $C_{24}H_{17}N_3O_2$ $(M+H)^+$ *m/z*: 380.1, measured 380.1.



N-(2-(5-(furan-2-yl)-1H-pyrazol-3-yl)phenyl)furan-2-carboxamide (1l) ^{1}H-NMR (400 MHz, acetone-d6) δ 8.83 (d, J=4.31 Hz, 1H), 7.89 (dd, J=7.82 Hz, 1.50 Hz, 1H), 7.73 (ddd, J=4.60 Hz, 1.70 Hz, 0.78 Hz, 2H), 7.36 (app t, 1H), 7.26 (dd, J=3.48 Hz, 0.76 Hz, 1H), 7.19 (app t, 1H), 7.15 (s, 1H), 6.95 (dd, J=3.40 Hz, 0.50 Hz, 1H), 6.65 (dd, J=3.48 Hz, 1.76 Hz, 1H), 6.63 (dd, J=3.42 Hz, 1.82 Hz, 1H); ^{13}C-NMR (100 MHz, acetone-d6) δ 157.1, 149.8, 145.9, 145.6, 144.0, 137.4, 136.5, 129.2, 128.8, 124.2, 121.3, 121.1, 115.0, 113.0, 112.7, 108.4, 100.9; LRMS calculated for $C_{18}H_{13}N_3O_3$ $(M+H)^+$ *m/z*: 320.1, measured 320.1.

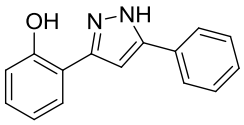


2-(5-(thiophen-2-yl)-1H-pyrazol-3-yl)phenol (2a) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.77 (dd, J=7.74 Hz, 1.54 Hz, 1H), 7.56 (br s, 1H), 7.24 - 7.15 (m, 2H), 7.11 (s, 1H), 6.93 (app q, 2H) ^{13}C-NMR (150 MHz, acetone-d6) δ 157.3, 153.5, 139.2, 130.0, 128.9, 127.4, 127.0, 119.3, 117.5, 116.8, 99.5; LRMS calculated for $C_{13}H_{10}N_2OS$ $(M+H)^+$ *m/z*: 243.1, measured 243.1.

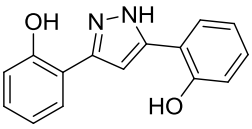


2-(5-(furan-3-yl)-1H-pyrazol-3-yl)phenol (2b) ^{1}H-NMR (400 MHz, acetone-d6) δ 8.13 (s, 1H), 7.73 – 7.69 (m, 2H), 7.20 (app tr, 1H), 7.07 (s, 1H), 6.96 – 6.87 (m, 3H); ^{13}C-NMR (100 MHz, acetone-d6) δ 157.1, 145.2, 141.0,

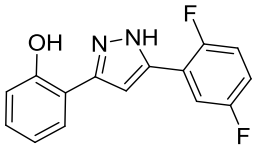
129.9, 127.5, 120.0, 117.7, 117.5, 109.4, 100.0; LRMS calculated for $C_{13}H_{10}N_2O_2$ $(M+H)^+$ *m/z*: 227.1, measured 227.1.



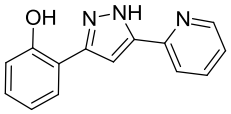
2-(5-phenyl-1H-pyrazol-3-yl)phenol (2c) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.91 - 7.87 (m, 2H), 7.78 (dd, J=7.74 Hz, 1.62 Hz, 1H), 7.52 (t, J=7.54 Hz, 2H), 7.43 (t, J=7.38 Hz, 1H), 7.27 (s, 1H), 7.21 (app t, 1H), 6.97 - 6.90 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d6) δ 157.2, 153.8, 144.7, 130.0, 129.9, 129.7, 127.6, 126.5, 120.0, 117.7, 117.5, 100.1; LRMS calculated for $C_{15}H_{12}N_2O$ $(M+H)^+$ *m/z*: 237.1, measured 237.1.



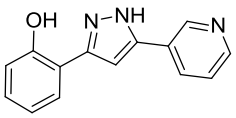
2,2'-(1H-pyrazole-3,5-diyl)diphenol (2d) ^{1}H-NMR (400 MHz, CD_3OD) δ 7.71 (d, J=7.52 Hz, 2H), 7.19 (t, J=7.58 Hz, 2H), 7.14 (s, 1H), 6.97 – 6.89 (m, 4H); ^{13}C-NMR (100 MHz, CD_3OD) δ 156.4, 130.2, 128.3, 120.7, 117.8, 117.4, 100.4; LRMS calculated for $C_{15}H_{12}N_2O_2$ $(M+H)^+$ *m/z*: 253.1, measured 253.2.



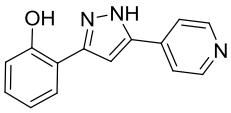
2-(5-(2,5-difluorophenyl)-1H-pyrazol-3-yl)phenol (2f) ^{1}H-NMR (600 MHz, acetone-d6) δ 9.12 (br s, 1H), 8.62 (br s, 1H), 8.24 (dd, J=7.89 Hz, 1.88 Hz, 1H), 7.79 (dd, J=7.74 Hz, 1.56 Hz, 1H), 7.51 (dd, J=7.59 Hz, 4.83 Hz, 1H), 7.38 (s, 1H), 7.23 (app t, 1H), 6.97 (d, J=8.10 Hz, 1H), 6.94 (t, J=7.30 Hz, 1H); ^{13}C-NMR (150 MHz, acetone-d6) δ 156.9, 150.5, 147.8, 133.5, 130.1, 129.9, 128.1, 127.8, 124.7, 120.2, 118.5, 117.6,117.5, 100.8; LRMS calculated for $C_{15}H_{10}F_2N_2O$ $(M+H)^+$ *m/z*: 273.1, measured 273.1.



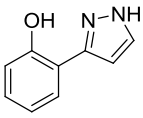
2-(5-(pyridin-2-yl)-1H-pyrazol-3-yl)phenol (2g) ^{1}H-NMR (400 MHz, acetone-d6) δ 10.93 (br s, 1H), 8.66 (d, J=4.64 Hz, 1H), 8.00 (m, 2H), 7.78 (dd, J=7.72 Hz, 1.56 Hz, 1H), 7.47 (s, 1H), 7.39 (app t, 1H), 7.22 (app t, 1H), 6.97 – 6.90 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d6) δ 150.5, 138.2, 129.9, 127.5, 124.4, 121.3, 120.0, 117.7, 117.6,100.8; LRMS calculated for $C_{1e}H_{11}N_3O$ $(M+H)^+$ *m/z*: 238.1, measured 238.1.



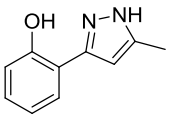
2-(5-(pyridin-3-yl)-1H-pyrazol-3-yl)phenol (2h) ^{1}H-NMR (400 MHz, acetone-d6) δ 9.12 (s, 1H), 8.62 (br s, 1H), 8.24 (dt, J= 7.96 Hz, 1.92 Hz, 1H), 7.80 (dd, J=7.76 Hz, 1.60 Hz, 1H), 7.51 (br, 1H), 7.38 (br s, 1H), 7.25 – 7.20 (m, 1H), 7.01 – 6.91 (m, 2H); ^{13}C-NMR (150 MHz, DMSO-d6) δ 148.3, 146.4, 132.2, 129.2, 127.5, 123.9, 116.3, 101.4; LRMS calculated for $C_{1e}H_{11}N_3O$ $(M+H)^+$ *m/z*: 238.1, measured 238.1.



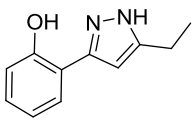
2-(5-(pyridin-4-yl)-1H-pyrazol-3-yl)phenol (2i) ^{1}H-NMR (600 MHz, DMSO-d6) δ 8.62 (d, J=4.80 Hz, 2H), 7.81 (app d, 2H), 7.72 (d, J=7.32, 1H), 7.39 (br, 1H), 7.20 (app t, 1H), 6.99 (d, J=7.80 Hz, 1H), 6.92 (app t, 1H); ^{13}C-NMR (150 MHz, DMSO-d6) δ 154.5, 150.2, 129.3,127.3, 119.5, 119.4, 116.4, 102.2; LRMS calculated for $C_{1e}H_{11}N_3O$ $(M+H)^+$ *m/z*: 238.1, measured 238.1.



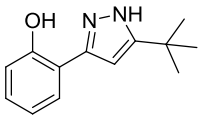
2-(1H-pyrazol-3-yl)phenol (2j) ^{1}H-NMR (400 MHz, acetone-d6) δ 11.01 (s, 1H), 7.90 (d, J=2.36 Hz, 1H), 7.72 (d, J=7.64 Hz, 1H), 7.19 (t, J=7.64 Hz, 1H), 6.96 – 6.85 (m, 3H); ^{13}C-NMR (100 MHz, acetone-d6) δ 157.0, 130.7, 129.7, 127.4, 127.2, 119.9, 117.9, 117.5, 102.3; LR7MS calculated for $C_9H_8N_2O$ $(M+H)^+$ *m/z*: 161.1, measured 161.2.



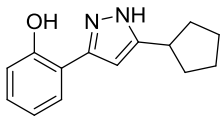
2-(5-methyl-1H-pyrazol-3-yl)phenol (2k) ^{1}H-NMR (600 MHz, acetone-d6) δ 11.07 (s, 1H), 7.63 (dd, J=7.74 Hz, J=1.50 Hz, 1H), 7.16 (t, J=7.50 Hz, 1H), 6.89 (d, J=8.16 Hz, 1H), 6.86 (t, J=7.50 Hz, 1H), 6.58 (s, 1H), 2.39 (s, 3H); ^{13}C-NMR (150 MHz, acetone-d6) δ 157.2, 129.5, 127.3, 119.8, 118.0, 117.4, 101.6, 10.6; LRMS calculated for $C_{10}H_{10}N_2O$ $(M+H)^+$ *m/z*: 175.1, measured 175.2.



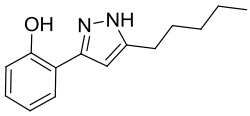
2-(5-ethyl-1H-pyrazol-3-yl)phenol (2l) ^{1}H-NMR (600 MHz, acetone-d6) δ 12.13 (br s, 1H), 11.09 (br s, 1H), 7.56 (J=7.70 Hz, 1.58 Hz, 1H), 7.16 (app tr, 1H), 6.87 (app q, 1H), 6.62 (s, 1H), 2.79 (q, J=7.60 Hz, 2H), 1.32 (t, J=7.60 Hz, 3H); ^{13}C-NMR (150 MHz, acetone-d6) δ 157.2, 152.8, 147.4, 129.5, 127.3, 119.8, 118.1, 117.4, 117.3, 100.1, 19.3, 13.7; LRMS calculated for $C_{11}H_{12}N_2O$ $(M+H)^+$ *m/z*: 189.1, measured 189.2.



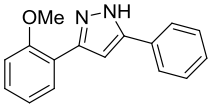
2-(5-(tert-butyl)-1H-pyrazol-3-yl)phenol (2m) ^{1}H-NMR (600 MHz, acetone-d6) δ 7.68 (dd, J=7.72 Hz, 1.60 Hz, 1H), 7.16 (app t, 1H), 6.91 - 6.84 (m, 1H), 6.65 (s, 1H), 1.42 (s, 9H); ^{13}C-NMR (150 MHz, acetone-d6) δ 157.2, 155.0, 152.4, 129.4, 127.3, 119.6, 118.1, 117.4, 117.3, 98. 6, 31.7, 30.4; LRMS calculated for $C_{13}H_{16}N_2O$ $(M+H)^+$ *m/z*: 217.1, measured 217.1.



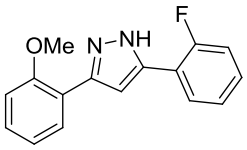
2-(5-cyclopentyl-1H-pyrazol-3-yl)phenol (2n) ^{1}H-NMR (400 MHz, acetone-d6) δ 12.13 (br, 1H), 11.09 (s, 1H), 7.66 (dd, J=7.72 Hz, 1.60 Hz, 1H), 7.16 (app t, 1H), 6.91 – 6.83 (m, 1H), 6.64 (s, 1H), 3.22 (p, J=7.98 Hz, 1H), 2.19 – 2.10 (m, 2H), 1.85 – 1.66 (m, 6H); ^{13}C-NMR (150 MHz, acetone-d6) δ 157.4, 152.7, 150.2, 129.5, 127.4, 119.6, 118.2, 117.8, 99.5, 33.8, 26.3, 25.7; LRMS calculated for $C_{14}H_{16}N_2O$ $(M+H)^+$ *m/z*: 229.1, measured 229.2.



2-(5-pentyl-1H-pyrazol-3-yl)phenol (2o) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.65 (dd, J=7.70 Hz, 1.56 Hz, 1H), 7.17 (app t, 1H), 6.91 – 6.84 (m, 1H), 6.62 (m, 1H), 2.76 (t, J=7.66 Hz, 2H), 1.74 (br p, 2H), 1.40 – 1.33 (m, 4H), 0.90 (t, J=7.06 Hz, 3H) ^{13}C-NMR (150 MHz, acetone-d6) δ 157.2, 152.9, 146.0, 129.5, 127.3, 119.8, 118.1, 117.4, 100.6, 41.4, 32.1, 25.9, 23.0, 14.2; LRMS calculated for $C_{14}H_{18}N_2O$ $(M+H)^+$ *m/z*: 231.2, measured 231.2.

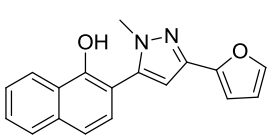


3-(2-methoxyphenyl)-5-phenyl-1H-pyrazole (3a) ^{1}H-NMR (400 MHz, acetone-d6) δ 7.92 (app d, 2H), 7.87 (app d, 1H), 7.41 (t, J=7.64 Hz, 2H), 7.38 – 7.27 (m, 2H), 7.20 – 7.15 (m, 2H), 7.06 (app t, 1H), 4.04 (s, 3H); ^{13}C-NMR (150 MHz, acetone-d6) δ 157.1, 130.1, 129.4, 128.7, 128.2, 126.2, 121.8, 112.6, 101.6, 56.0; LRMS calculated for $C_{16}H_{14}N_2O$ $(M+H)^+$ *m/z*: 251.1, measured 251.2.

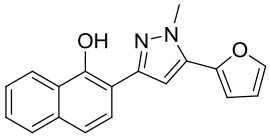


5-(2-fluorophenyl)-3-(2-methoxyphenyl)-1H-pyrazole (3b) ^{1}H-NMR (400 MHz, acetone-d6) δ 8.09 (br, 1H), 7.85 (d, J=6.84 Hz, 1H), 7.39 – 7.33 (m, 2H), 7.28 – 7.17 (m, 3H), 7.16 (d, J=3.60 Hz, 1H), 7.07 (t, J=7.47 Hz, 1H), 4.04 (s, 3H); ^{13}C-NMR (150 MHz, acetone-d6); δ 157.1, 130.2, 130.0, 129.8, 129.2, 129.1,

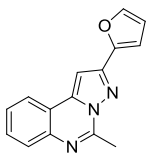
128.7, 125.2, 121.9, 116.9, 112.6, 104.7, 56.0; LRMS calculated for $C_{16}H_{13}FN_2O$ $(M+H)^+$ *m/z*: 269.1, measured 269.1.



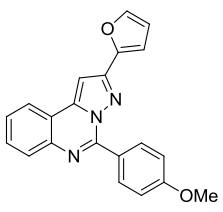
2-(3-(furan-2-yl)-1-methyl-1H-pyrazol-5-yl)naphthalen-1-ol (4a) ^{1}H-NMR (400 MHz, acetone-d6) δ 8.66 (br, 1H), 8.40 – 8.36 (m, 1H), 7.95 – 7.90 (m, 1H), 7.62 – 7.52 (m, 4H), 7.34 (d, J=8.44 Hz, 1H), 6.70 (d, J=3.80 Hz, 1H), 6.58 (s, 1H), 6.54 (dd, J=3.28 Hz, 1.80 Hz, 1H), 3.77 (s, 3H); ^{13}C-NMR (150 MHz, acetone-d6) δ 151.6, 150.6, 143.7, 142.5,141.4, 136.1, 128.8, 128.6, 127.9, 126.5, 126.2, 123.4, 120.6, 112.1, 111.9, 105.6, 104.9, 37.5; LRMS calculated for $C_{18}H_{15}N_2O_2$ $(M+H)^+$ *m/z*: 291.1, measured 291.2.



2-(5-(furan-2-yl)-1-methyl-1H-pyrazol-3-yl)naphthalen-1-ol (4b) ^{1}H-NMR (600 MHz, acetone-d6) δ 8.36 – 8.33 (m, 1H), 7.84 – 7.80 (m, 2H), 7.50 (dt, J=9.72 Hz, 3.36 Hz, 2H), 7.45 (d, J=8.52 Hz, 1H), 7.17 (s, 1H), 6.98 (d, J=3.78 Hz, 1H), 6.69 (dd, J=3.42 Hz, 1.80 Hz, 1H), 4.21 (s, 3H); ^{13}C-NMR (150 MHz, acetone-d6) δ 152.6, 151.4, 145.0, 144.6, 136.2, 135.1, 128.3, 127.4, 126.3,126.0, 124.9, 123.5, 119.7, 112.7, 110.7, 110.4, 102.1, 39.2; LRMS calculated for $C_{18}H_{15}N_2O_2$ $(M+H)^+$ *m/z*: 291.1, measured 291.2.

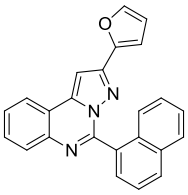


2-(furan-2-yl)-5-methylpyrazolo[1,5-c]quinazoline (5a) ^{1}H-NMR (400 MHz, acetone-d6) δ 8.24 (app dd, 1H), 7.86 (d, J=7.61 Hz, 1H), 7.65 – 7.64 (m, 1H), 7.71 (app td, 1H), 7.63 (app t, 1H), 7.49 (s, 1H), 7.06 (d, J=3.42 Hz, 1H), 6.65 (dd, J=3.36 Hz, 1.80 Hz, 1H), 2.94 (s, 3H); ^{13}C-NMR (150 MHz, acetone-d6) δ 149.1, 148.9, 147.5, 144.3, 140.9, 140.7, 130.6, 128.7, 127.9, 124.2, 120.0, 112.4, 109.3, 96.1, 95.9, ; LRMS calculated for $C_{15}H_{11}N_3O$ $(M+H)^+$ *m/z*: 250.1, measured 250.1.



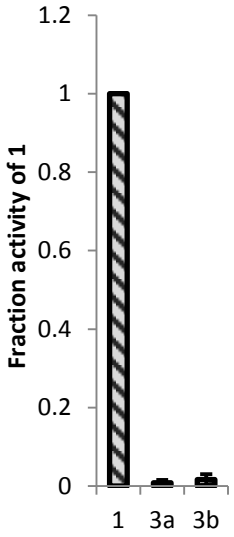
2-(furan-2-yl)-5-(4-methoxyphenyl)pyrazolo[1,5-c]quinazoline (5b) ^{1}H-NMR (400 MHz, acetone-d6) δ 8.69 (d, J=9.04 Hz, 2H), 8.28 (dd, J=7.88 Hz, 1.12 Hz, 1H), 7.96 (d, J=8.16 Hz, 1H), 7.77 – 7.71 (m, 2H), 7.64 (app t, 1H),

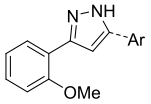
7.56 (s, 1H), 7.15 (d, J=9.04 Hz, 2H), 7.07 (d, J=3.33 Hz, 1H), 6.65 (dd, J=3.36 Hz, 1.80 Hz, 1H), 3.95 (s, 3H); ^{13}C-NMR (100 MHz, acetone-d6) δ 163.0, 147.5, 142.7, 141.0, 133.5, 130.9, 129.2, 128.3, 124.2, 114.1, 112.7, 109.5, 96.0, 55.9; LRMS calculated for $C_{21}H_{15}N_3O_2$ $(M+H)^+$ *m/z*: 342.1, measured 342.1.



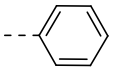
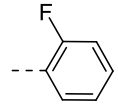
2-(furan-2-yl)-5-(naphthalen-1-yl)pyrazolo[1,5-c]quinazoline (5c) ^{1}H-NMR (600 MHz, acetone-d6) δ 8.16 (d, J=7.80 Hz, 1H), 8.07 (app dd, 2H), 7.96 (app dd, 2H), 7.75 – 7.64 (m, 4H), 7.52 (t, J=7.50 Hz, 1H), 7.49 (s, 1H), 7.42 (t, J=7.62 Hz, 1H), 7.35 (s, 1H), 6.79 (d, J=3.18 Hz, 1H), 6.45 (br s, 1H); ^{13}C-NMR (150 MHz, acetone-d6) δ 148.5, 147.8, 147.7, 143.4, 141.3, 139.6, 133.9, 131.5, 131.2, 130.4, 129.8, 128.7, 128.6, 128.5, 128.4, 127.0, 126.4, 125.4, 125.1, 123.4, 119.6, 111.9, 109.3, 95.9; LRMS calculated for $C_{24}H_{15}N_3O$ $(M+H)^+$ *m/z*: 362.1, measured 362.1.

5. Supplemental Figure 1: Activation of HssRS by 3a and 3b at 50 μM. Activity is presented as the fraction of activation by the indicated derivative relative to **1** at 50 μM. Data are the average of three replicates. Error bars represent one standard deviation from the mean.

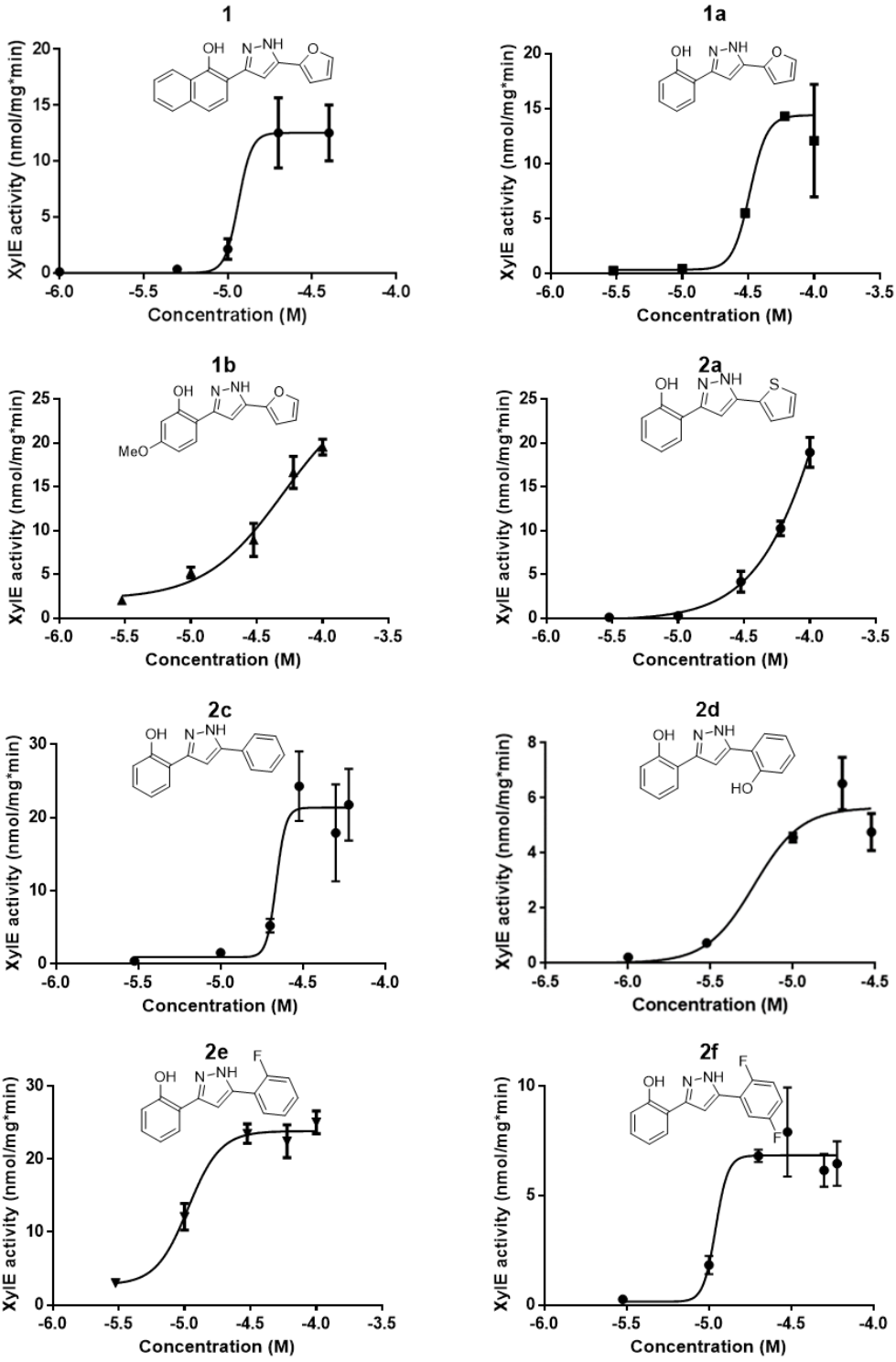




3a,3b

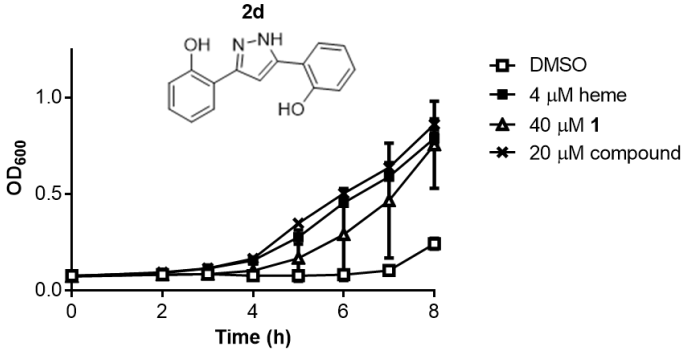
Cmpd	Aryl Group
3a	
3b	

6. Supplemental Figure 2. Concentration response curves for **1** and the seven most active derivatives; **1a**, **1b**, **2a**, **2c**, **2e**, and **2f**. Data were collected using the XylE assay and are the average of three replicates. Error bars are one stardard deviation from the mean.

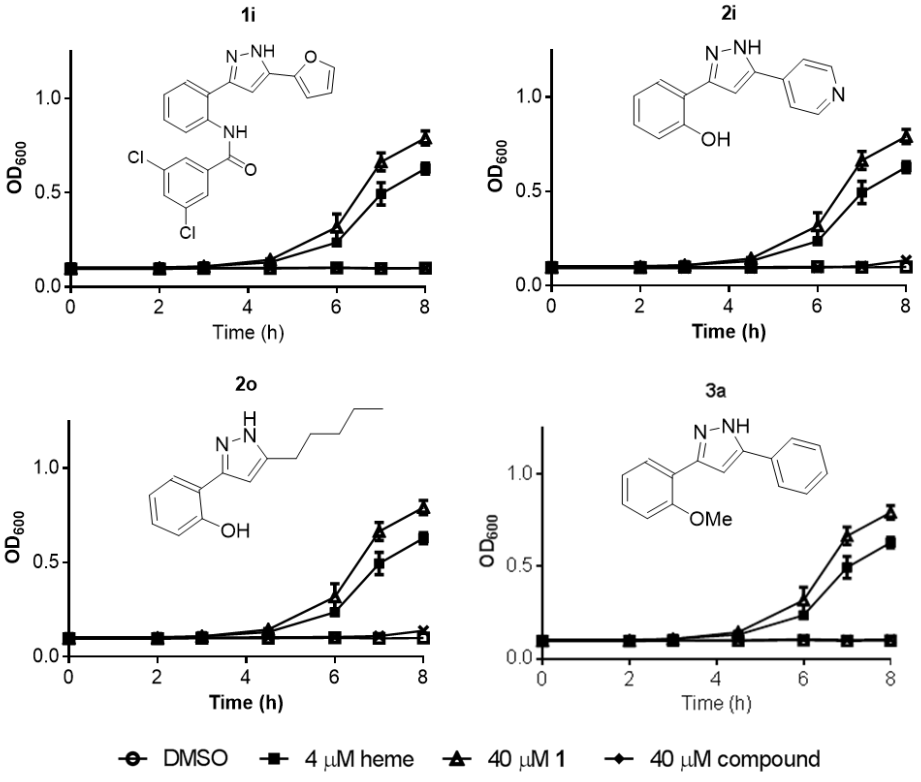


7. Supplemental Figure 3. Adaptation for heme toxicity by **a**) compound **2d** at the nontoxic concentration of 20 μM and **b**) compounds determined to be inactive based on single point XylE data. Overnight cultures were grown in medium with the indicated additive and then subcultured into medium containing a toxic concentration of heme (20 μM). Growth was monitored by reading OD_{600} at indicated time points. Data are the average of three replicates and error bars represent one standard deviation from the mean.

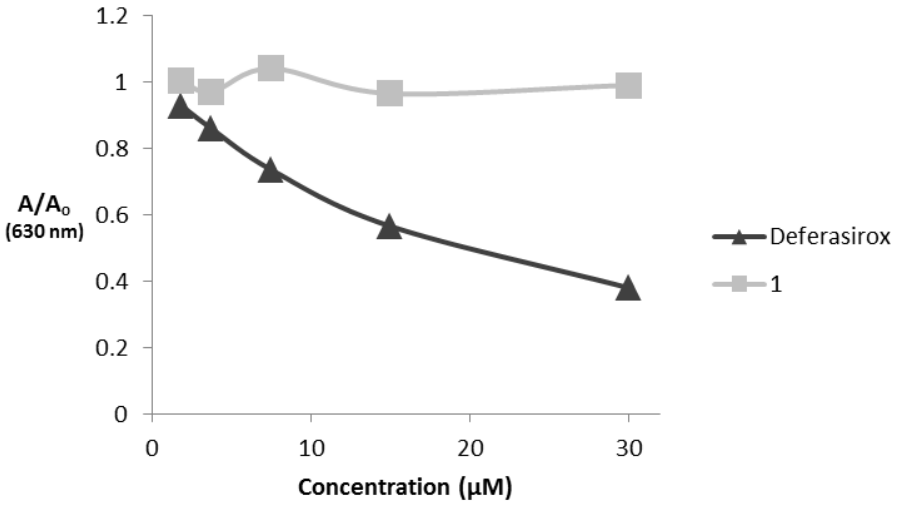
a.

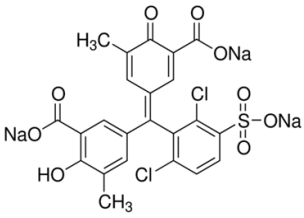


b.

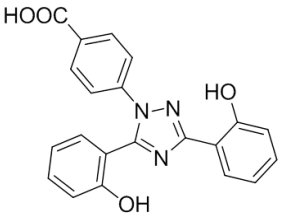


8. Supplemental Figure 4. CAS assay[3] to determine the ability of **1** to bind iron. Deferasirox[4] was used as a positive control.





Chromoazural S



Deferasirox

9. References

(1) Kallander, L. S., Lu, Q., Chen, W., Tomaszek, T., Yang, G., Tew, D., Meek, T. D., Hofmann, G. A., Schulz-Pritchard, C. K., Smith, W. W., Janson, C. A., Ryan, M. D., Zhang, G.-F., Johanson, K. O., Kirkpatrick, R. B., Ho, T. F., Fisher, P. W., Mattern, M. R., Johnson, R. K., Hansbury, M. J., Winkler, J. D., Ward, K. W., Veber, D. F., and Thompson, S. K. (2005) 4-Aryl-1,2,3-triazole: a novel template for a reversible methionine aminopeptidase 2 inhibitor, optimized to inhibit angiogenesis in vivo. *J. Med. Chem. 48*, 5644–5647.

(2) Mike, L. A., Dutter, B. F., Stauff, D. L., Moore, J. L., Vitko, N. P., Aranmolate, O., Kehl-Fie, T. E., Sullivan, S., Reid, P. R., DuBois, J. L., Richardson, A. R., Caprioli, R. M., Sulikowski, G. A., and Skaar, E. P. (2013) Activation of heme biosynthesis by a small molecule that is toxic to fermenting Staphylococcus aureus. *Proc. Natl. Acad. Sci. U. S. A. 110*, 8206–8211.

(3) Schwyn, B., and Neilands, J. B. (1987) Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem. 160*, 47–56.

(4) Reed, C., Ibrahim, A., Edwards, J. E., Walot, I., and Spellberg, B. (2006) Deferasirox, an iron-chelating agent, as salvage therapy for rhinocerebral mucormycosis. *Antimicrob. Agents Chemother. 50*, 3968–3969.