Supplemental Methods

General procedure for the preparation of compound 1

To a stirred solution of anthranilamide (2.791 g, 20.5 mmol) in DMA (30 mL) was added *n*-octanal (3.2 mL, 20.5 mmol) and sodium bisulfite (3.2 g, 30.8 mmol). The resulting reaction mixture was allowed to stir at reflux for 2 hr. The reaction mixture was poured into water (500 mL) and the precipitate filtered. The precipitate was recrystallized from EtOH to yield **1** as an off-white crystalline solid (3.82 g, 76%).

General procedure for the preparation of compound 2

Et_3N (1.76 mL, 12 mmol) was added to a suspension of anthranilic acid (823 mg, 6 mmol) in DCM (20 mL). The mixture was cooled on ice and octanoyl chloride (1.02 mL, 6 mmol) was added dropwise. The reaction mixture was then stirred at room temperature for 2.5 hr. After 2.5 hr the reaction mixture was cooled on ice. Et_3N (0.92 mL, 6.6 mmol) and ethyl chloroformate (0.63 mL, 6.6 mmol) were added dropwise to the mixture successively. The reaction mixture was then stirred at 0ºC for 1 hr and at room temperature for 1.5 hr. The reaction mixture was concentrated *in vacuo* to yield 2-heptyl-4*H*-benzo[*d*][1,3]oxazin-4-one as a pale yellow crystalline solid, which was carried through to the next step without further purification.

2-heptyl-4*H*-benzo[*d*][1,3]oxazin-4-one was dissolved in pyridine (15 mL). Hydrazine hydrate (0.37 mL, 12 mmol) was added and the reaction mixture was refluxed for 1 hr. Then the mixture was left to cool to room temperature. Conc. HCl (15 mL) and ice (15 g) were added to the mixture which was left to stand at room temperature overnight. A solid crashed out and was collected by vacuum filtration and washed with water. The solid was then recrystallised from ethanol to yield compound **2** as a colorless solid (52 mg, 3%).

General procedure for the preparation of compounds 3-14, 16 and 17

Methyl-3-oxodecanoate

2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (18.7 g, 130 mmol) was dissolved in distilled dichloromethane (200 mL). The solution was cooled to 0°C under a N_2 atmosphere. To the cooled solution were added pyridine (20.5 mL, 260 mmol) and octanoyl chloride (23.8 mL, 140 mmol), drop-wise. The solution was stirred at 0°C for 1 hr and then at room temperature for 1 hr. The mixture was washed with 5% HCl (3 x 75 mL) and with distilled water (75 mL). The solution was then dried with anhydrous $MgSO_4$ filtered and concentrated *in vacuo* to yield acyl Meldrum's acid as a brown oil which was used in the subsequent step without further purification.

Acyl Meldrum's acid was dissolved in MeOH (180 mL) and heated at reflux for 5 hr with constant stirring. After allowing to cool, the reaction mixture was concentrated *in vacuo* yielding the crude product as an orange oil. Purification was achieved by fractional distillation affording the β-keto ester as a pale yellow oil (16.7 g, 64 % yield).

Methyl-3-oxododecanoate

2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (18.7 g, 130 mmol) was dissolved in distilled dichloromethane (200 mL). The solution was cooled to 0°C under a N_2 atmosphere. To the cooled solution were added pyridine (20.5 mL, 260 mmol) and decanoyl chloride (28.9 mL, 140 mmol), drop-wise. The solution was stirred at 0°C for 1 hr and then at room temperature for 1 hr. The mixture was washed with 5% HCl (3 x 75 mL) and with distilled water (75 mL). The solution was then dried with anhydrous $MgSO_4$ filtered and concentrated *in vacuo* to yield acyl Meldrum's acid as a brown oil which was used in the subsequent step without further purification.

Acyl Meldrum's acid was dissolved in MeOH (180 mL) and heated at reflux for 5 hr with constant stirring. After allowing to cool, the reaction mixture was concentrated *in vacuo* yielding the crude product as a brown oil. Purification was achieved by fractional distillation affording the β-keto ester as a yellow oil (17.5 g, 59 % yield).

Substituted 2-alkyl-4-quinolones

To a solution of the β-ketoester (5 mmol) in dry hexane (10 mL) were added the substituted aniline (5 mmol) and *p*-toluene sulfonic acid (0.1 mmol). The reaction mixture was heated at reflux (>70°C) under N_2 atmosphere overnight using a Dean-Stark system. Upon completion, the reaction mixture was concentrated *in vacuo* to afford the crude β-enamino ester, which was then added drop-wise to refluxing diphenyl ether (2 mL, >260°C). Reflux was maintained for approx. 1.5 hr. After cooling to room temperature, ether (approx. 20 mL) was added to the reaction mixture and left overnight at 5°C, allowing the quinolone product to precipitate. The quinolone was collected by vacuum filtration, recrystallised from hot methanol (if necessary) and dried *in vacuo*.

General procedure for the preparation of compound 15

HHQ (0.3 g, 1.233 mmol) and phosphorus pentasulfide (0.1 g, 0.226 mmol) were added to a dry Schlenk tube and evacuated and refilled with nitrogen three times. Hexamethyldisiloxane (HMDO) (0.334 g, 0.44 ml, 2.059 mmol) and dry xylene (1 mL) were added. The mixture was heated to 140°C for 5 hr. The mixture was allowed to cool to room temperature, was poured into a conical flask and placed in an ice bath. K_2CO_3 (5.3 M, 0.4 mL) was added followed by acetone (0.5 mL). The mixture was stirred at 0°C for 30 min. The mixture was poured into water (10 mL), extracted with DCM (3 x 10 mL), washed with dilute K_2CO_3 (1 M), water, brine and then dried over $MgSO_4$. The solvent was evaporated *in vacuo* and dried on the high vacuum line giving the compound **15** as an orange solid (152 mg, 48%).

Analogues in desiccated form were re-suspended in Methanol to create a 10 mM stock. A working concentration of 100 µM was used in all experiments.

Biofilm formation.

C. albicans biofilm formation was carried out in 96 well plates, as previously described (Ramage et al., 2001). Briefly, *C. albicans* was inoculated into Yeast Nitrogen Base [10% (w/v)] and glucose/maltose [10% (w/v)] and incubated overnight at 30ºC. Seeding densities for all subsequent experiments (N=3) were OD_{600}=0.05. Cells were grown in Yeast Nitrogen Base supplemented with 1 M Phosphate buffer and acetyl-D –glucosamine (YNB-NP) along with appropriate volumes of analogues. Cells were cultured in 96 well plates for 1 hr at 37 °C to facilitate yeast attachment. After this incubation, the supernatants were aspirated, the wells washed twice with YNB-NP media, and fresh media with the same concentration of analogue added to the appropriate wells. The plate was incubated for 24 hr at 37°C. The next day, the cultures were aspirated and the wells washed once with YNB-NP media.

XTT Metabolic Assay.

C. albicans biofilm quantification was carried out in 96 well plates, as previously described using a semi-quantitative Tetrazolium salt, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H tetrazolium-5-carboxanilide inner salt (XTT) reduction assay (Hawser, 1996). XTT (0.01 g) was dissolved in sterile water (20 ml) and filter sterilized. 10 µl of Menadione dissolved in Acetone was added to the XTT solution, just before use. The XTT-Menadione Solution (100 µl) was added to each well. The plate was incubated in the dark at 37°C for up to 2 hr to allow for color development. The OD_{492nm} was recorded for each well. Experiments were repeated at least three times, with at least eight technical replicates.

Confocal Scanning Laser Microscopy.

C. albicans biofilms were grown on glass coverslips in 6 well plates, using the biofilm formation protocol above. Briefly, an overnight culture of *C. albicans* was added to YNB-NP media to give an OD_{600}=0.05. Analogues were added at appropriate concentrations. The 6 well plates were incubated at 37ºC for 1 hr to facilitate attachment after which they were washed once in YNB-NP and then fresh analogue in YNB-NP added. Plates were incubated overnight at 37ºC. Next day, glass coverslips were washed once in YNB-NP and stained. Calcofluor (1 mg/ml) and 10% (w/v) potassium hydroxide were added drop-wise to coverslips, washed in PBS and viewed. Concavalin A and FUN-1 were added at 50 µg/ml in 1 ml PBS and incubated at 37ºC for 30 mins. Coverslips were washed in PBS and viewed. All imaging was carried out on a Zeiss LSM5 confocal microscope. Confocal images were recorded under a bright field lens using x20 objective magnification. Filter cubes facilitating fluorescent imaging were used to record images for Calcofluor at 405 nm, Con A at 488 nm and FUN-1 at 543 nm. All images were captured using the Zeiss HBO-100 microscope illuminating system, processed using the Zen AIM application imaging program and converted to JPGs using Axiovision 40 Ver. 4.6.3.0. A minimum of three independent biological repetitions were carried out.

Supplemental Compound Data

3-Amino-2-heptylquinazolin-4(3*H*)-one (2)[2]

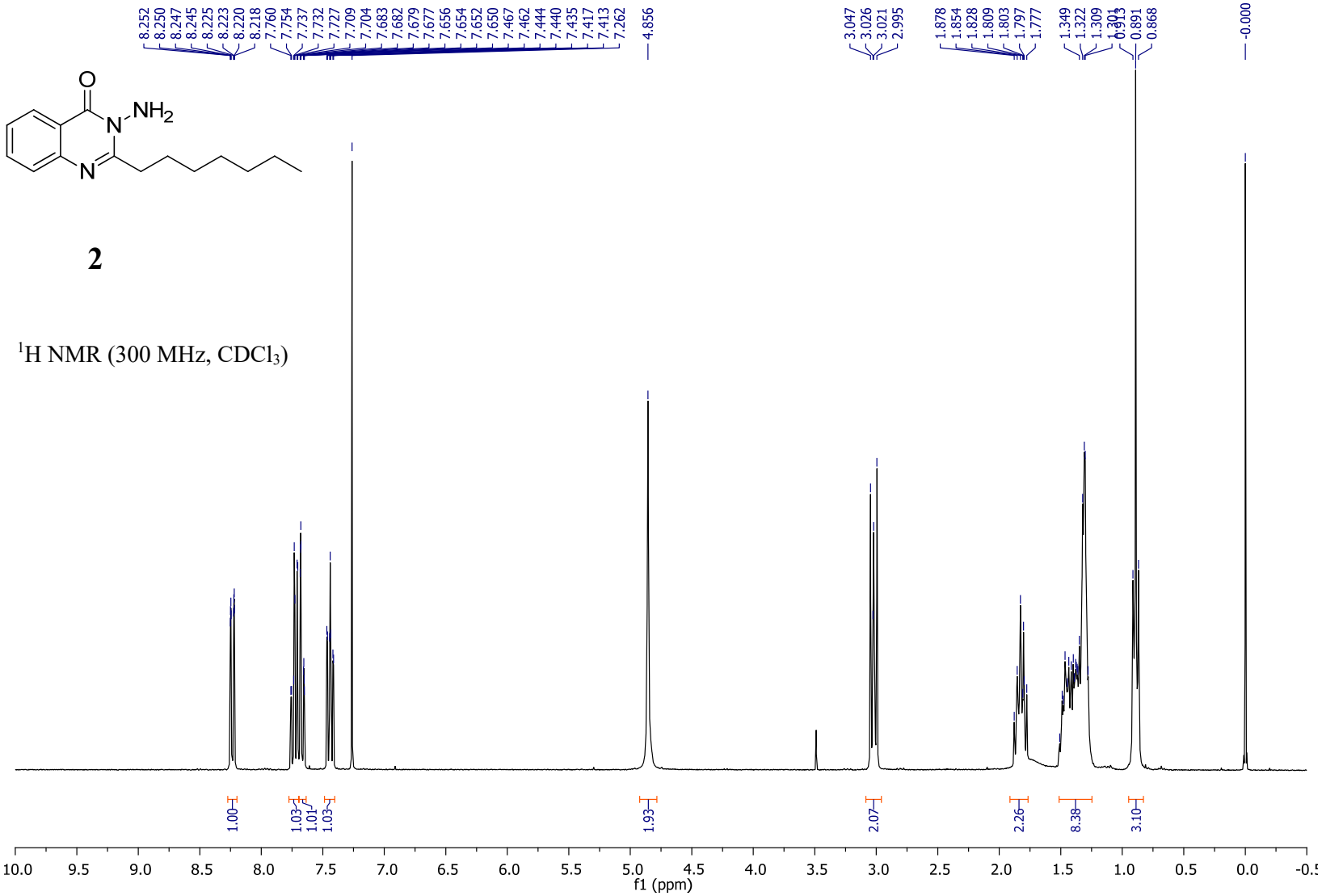
Colourless solid; yield: 52 mg (3 %); m. p. = 73-75 ºC (MeOH); IR (KBr): ν 3428, 3301, 3203, 1665, 1632, 1591 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, t, *J* = 6.8 Hz), 1.25-1.50 (8H, m), 1.75-1.90 (2H, m), 2.95-3.05 (2H, m), 4.86 (2H, s), 7.44 (1H, ddd, *J* = 8.2 and 6.8 Hz, ^{4}J = 1.4 Hz), 7.67 (1H, ddd, *J* = 8.3 Hz, ^{4}J = 1.4 Hz, ^{5}J = 0.5 Hz), 7.73 (1H, ddd, *J* = 8.3 and 6.8 Hz, ^{4}J = 1.5 Hz), 8.24 (1H, ddd, *J* = 8.2 Hz, ^{4}J = 1.5 Hz, ^{5}J = 0.5 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 14.1, 22.6, 27.2, 29.0, 29.4, 31.7, 34.6, 120.0, 126.2, 126.5, 127.2, 134.2, 147.1, 158.4, 161.9. HRMS calcd. (%) for $C_{15}H_{22}N_3O$: 260.1763; found: 260.1752. Elemental analysis calcd. (%) for $C_{15}H_{21}N_3O$: C = 69.47, H = 8.16, N = 16.20; found: C = 69.51, H = 8.23, N = 16.10.

7-Chloro-2-heptylquinolin-4(1*H*)-one and 5-chloro-2-heptylquinolin-4(1*H*)-one 9:1 (9)

^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.86 (2 x 3H, t, *J* = 6.7 Hz), 1.20-1.35 (2 x 8H, m), 1.60-1.70 (2 x 2H, m), 2.57 (2 x 2H, t, *J* = 7.7 Hz), 5.88 (1H, s), 5.94 (1H, s), 7.21 (1H, dd, *J* = 7.1 Hz, ^{4}J = 1.8 Hz), 7.29 (1H, dd, *J* = 8.6 Hz, ^{4}J = 2.0 Hz), 7.45-7.50 (2H, m), 7.55 (1H, d, ^{4}J = 2.0 Hz), 8.02 (1H, d, *J* = 8.6 Hz), 11.52 (2 x 1H, bs).

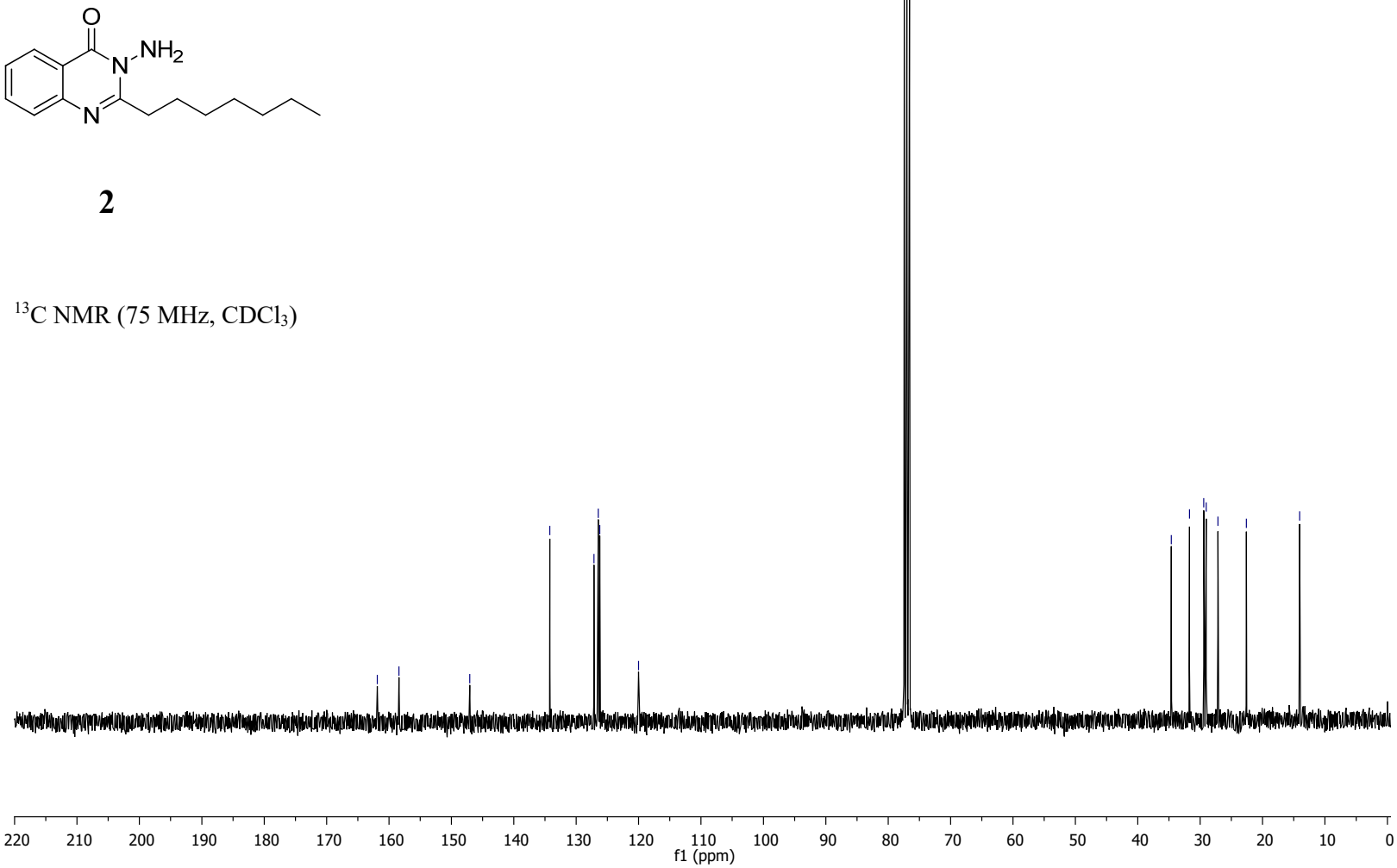
2-Heptylquinoline-4-thiol (13)

Orange solid; yield: 152 mg (48 %); m. p. = 61-62 ºC (EtOAc); IR (KBr): ν 3445, 1625, 1464, 1276 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (3H, t, *J* = 6.8 Hz), 1.10-1.30 (8H, m), 1.55-1.65 (2H, m), 2.60 (2H, t, *J* = 7.8 Hz), 7.30-7.40 (2H, m), 7.55-7.65 (2H, m), 8.89 (1H, d, *J* = 8.3 Hz), 10.60 (1H, bs); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 14.0, 22.5, 28.9, 29.0, 29.1, 31.5, 33.6, 118.7, 124.5, 125.8, 129.3, 131.6, 132.4, 136.0, 148.7. HRMS calcd. (%) for $C_{16}H_{22}NS$: 260.1473; found: 260.1459.



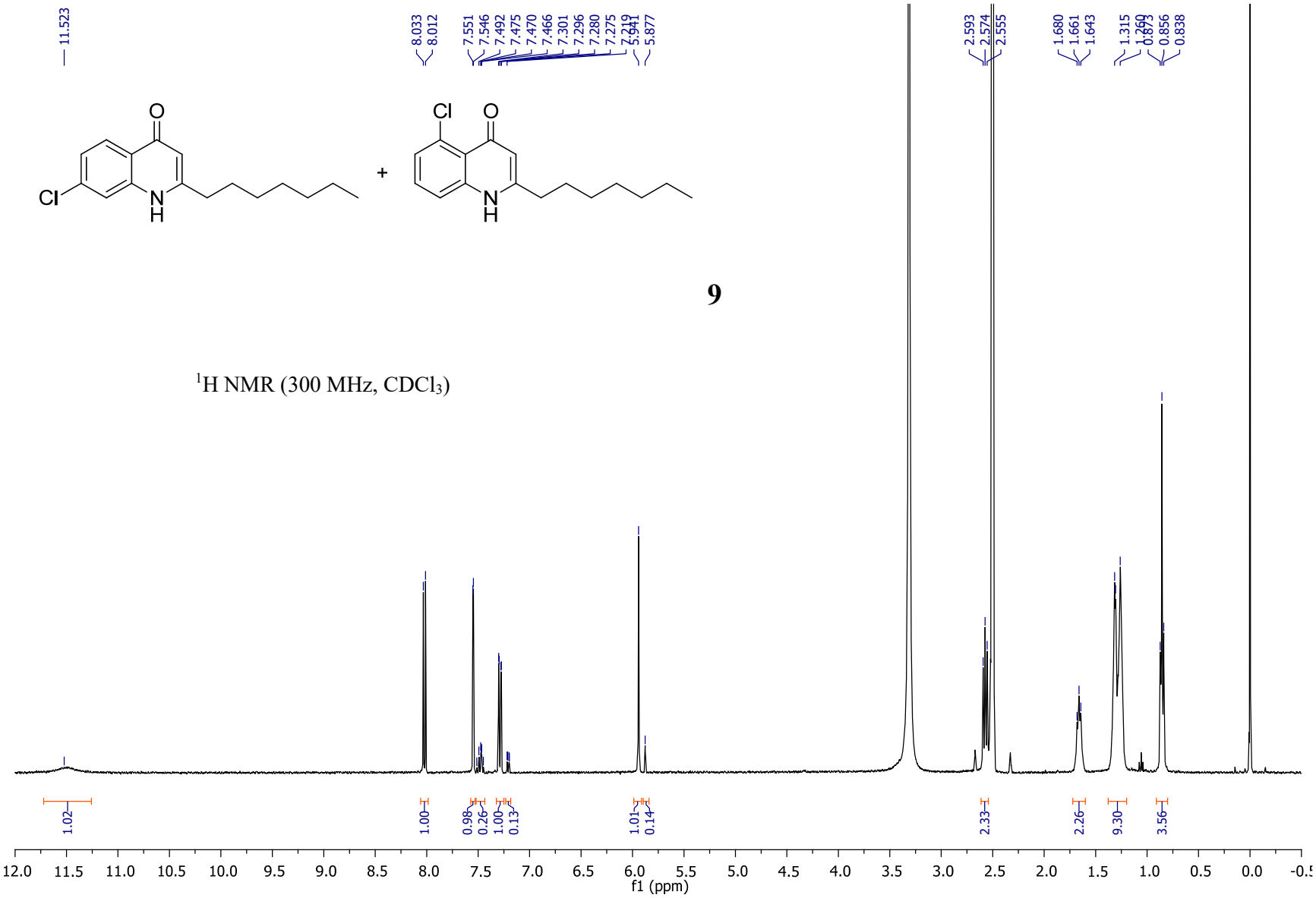
2

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



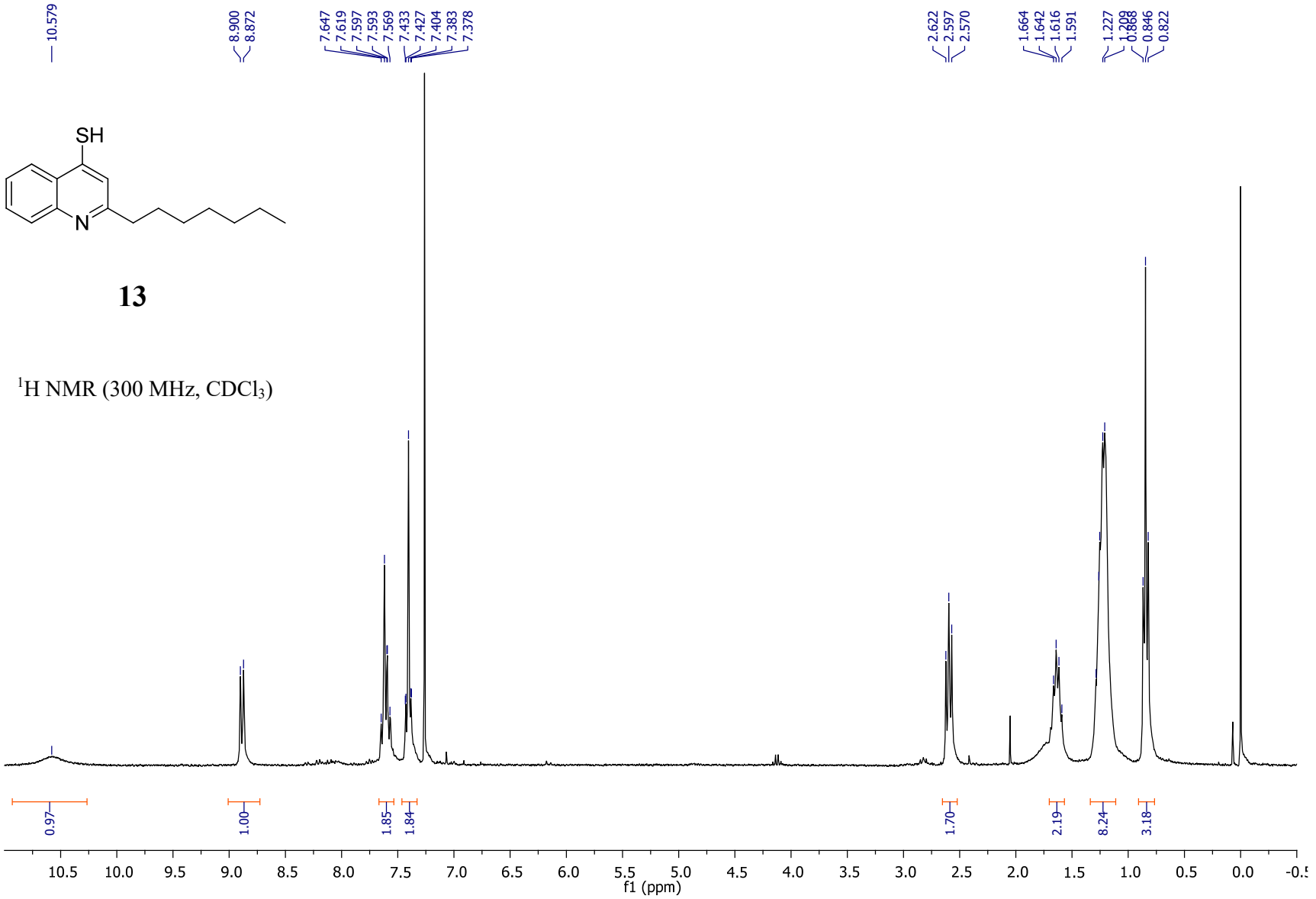
2

^{13}C NMR (75 MHz, $CDCl_3$)



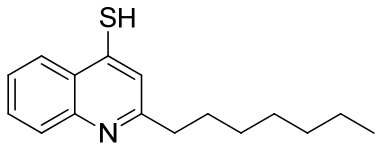
9

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



13

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



13

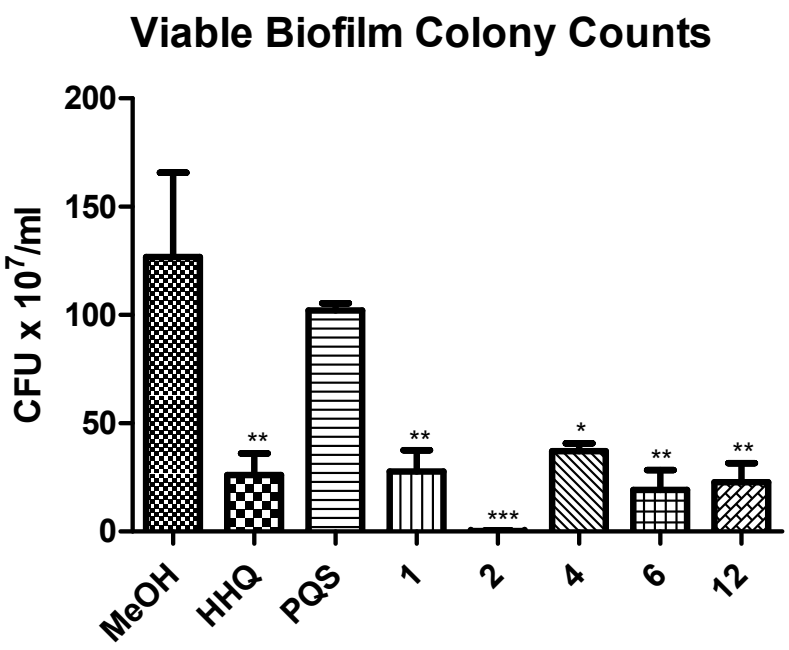
^{13}C NMR (75 MHz, $CDCl_3$)

Supplemental Table S1. Gene information and primers used in this study

Gene	Protein Function	Product Length (bp)	Sequence (5'- 3')	Probe*
ACT1	Multifunctional in several cell processes	68	Forward; accaccggtattgttttgga	9
			Reverse; agcgtaaattggaacaacgtg	
ALS3	Cell wall adhesin	60	Forward; tgaatattggtctcaatcatttgc	77
			Reverse; gtgtttcctggtggtccagt	
CPH1	Mating transcription factor	60	Forward; ataaaggcaataccgcaaactg	74
			Reverse; ttgtggttgaccaccattacc	
ECE1	Hyphal specific protein	63	Forward; tcaagctgccatcatccac	50
			Reverse; gcagctggagcaacatctct	
EFB1	Translation elongation factor	105	Forward; aaccacattgcttcattcactg	28
			Reverse; ttcgtcatcttcttcttcagca	
ESS1	Prolyl isomerase	90	Forward; gattgcagctcacatgacaga	157
			Reverse; caaattgaatgcggcttctt	
HWP1	Hyphal cell wall protein	65	Forward; ggatcccagatatcccagaaa	78
			Reverse; agttgtagcaggaatgtttggag	
RBT1	Cell wall protein	108	Forward; cagctaccgaatcagctcct	1
			Reverse; gggcaactggagtaactgga	
TUP1	Transcriptional corepressor	60	Forward; ggtcagtcagaatctgggactt	118
			Reverse; cttcgatcgacaaagtcaagg	
BCR1	Transcription factor	63	Forward; tcaatatcaaggtcctcaacca	10
			Reverse; tgctgaccaggtacttgttgtt	
DCK1	Putative guanine nucleotide exchange factor	60	Forward; tttgttgctagagtcgggaaa	18
			Reverse; atccaaaattgtgatataaccctca	
HGT2	Putative glucose transporter	68	Forward; tgcatcctttgcctttacttg	115
			Reverse; tgttgtcaccccagatttca	
ORF 19.2457	Protein of unknown function	63	Forward; tcgaccataattgtgatttgatct	140
			Reverse; tcaaatgagtctgtaatccacca	
IHD1	GPI-anchored protein	64	Forward; caccaataccgcatccact	129
			Reverse; cttcttagcagcgtcagaacc	

*Roche Universal ProbeLibrary (UPL) Probe Number
The annealing temperature of all primer sets is 58ºC
Protein Function; http://www.candidagenome.org

a


Viable Biofilm Colony Counts
CFU x 10^7/ml
200
150
100
50
0
**
**
***
*
**
**
MeOH
HHQ
PQS
1
2
4
6
12

b

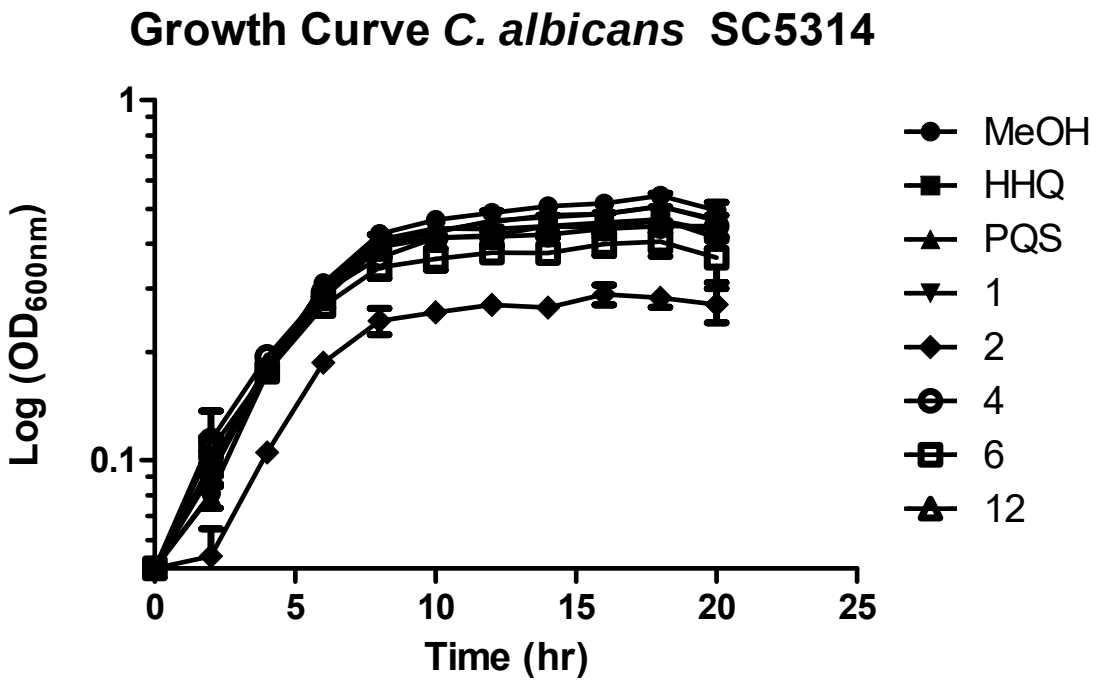

Growth Curve C. albicans SC5314
Log (OD600nm)
1
0.1
0
5
10
15
20
25
Time (hr)
MeOH
HHQ
PQS
1
2
4
6
12

Figure S1

(a)

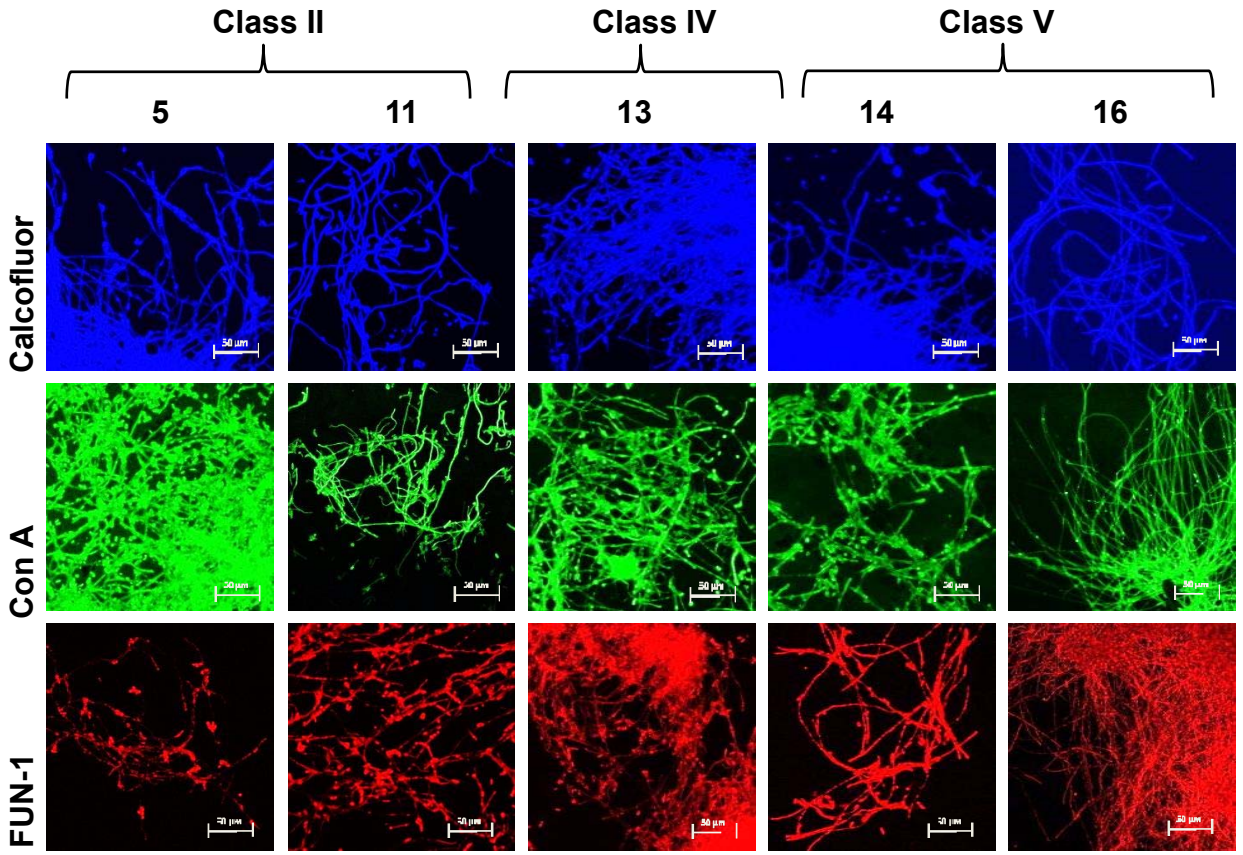


Figure S2

(b)

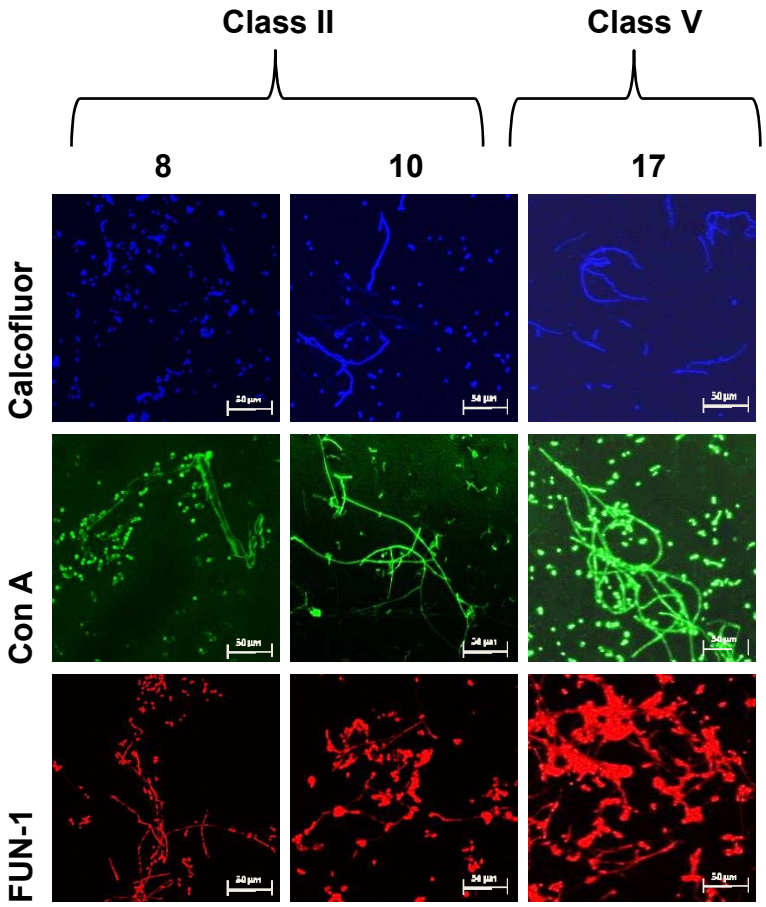


Figure S2

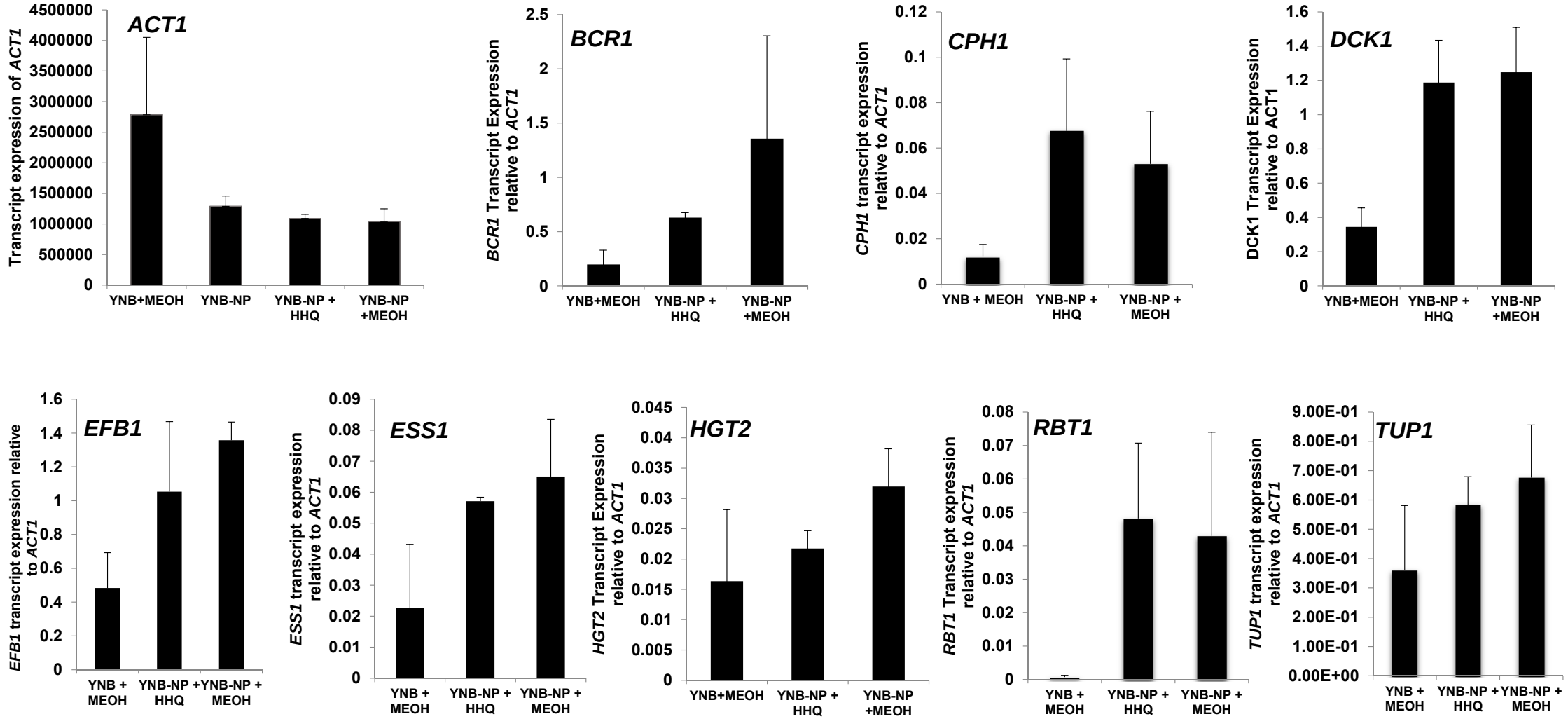

ACT1
Transcript expression of ACT1
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
YNB+MEOH
YNB-NP
YNB-NP + HHQ
YNB-NP +MEOH
BCR1
BCR1 Transcript Expression relative to ACT1
2.5
2
1.5
1
0.5
0
YNB+MEOH
YNB-NP + HHQ
YNB-NP +MEOH
CPH1
CPH1 transcript expression relative to ACT1
0.12
0.1
0.08
0.06
0.04
0.02
0
YNB + MEOH
YNB-NP + HHQ
YNB-NP + MEOH
DCK1
DCK1 Transcript Expression relative to ACT1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
YNB+MEOH
YNB-NP + HHQ
YNB-NP +MEOH
EFB1
EFB1 transcript expression relative to ACT1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
YNB + MEOH
YNB-NP + HHQ
YNB-NP + MEOH
ESS1
ESS1 transcript expression relative to ACT1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
YNB + MEOH
YNB-NP + HHQ
YNB-NP + MEOH
HGT2
HGT2 Transcript Expression relative to ACT1
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
YNB+MEOH
YNB-NP + HHQ
YNB-NP +MEOH
RBT1
RBT1 Transcript expression relative to ACT1
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
YNB + MEOH
YNB-NP + HHQ
YNB-NP + MEOH
TUP1
TUP1 transcript expression relative to ACT1
9.00E-01
8.00E-01
7.00E-01
6.00E-01
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
YNB + MEOH
YNB-NP + HHQ
YNB-NP + MEOH

Figure S3

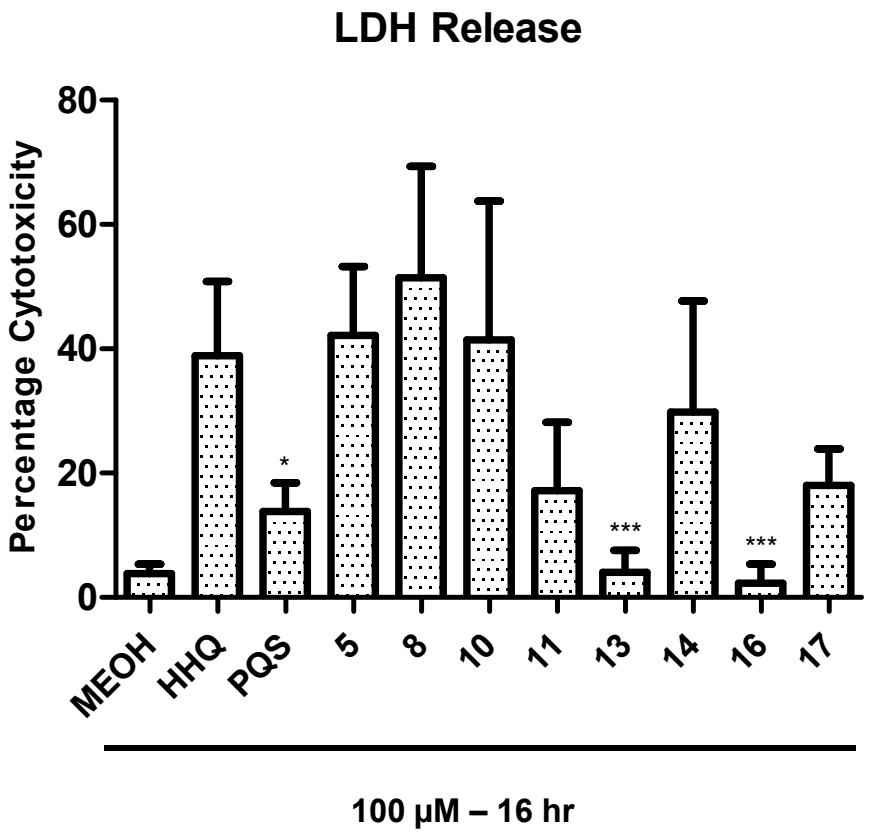
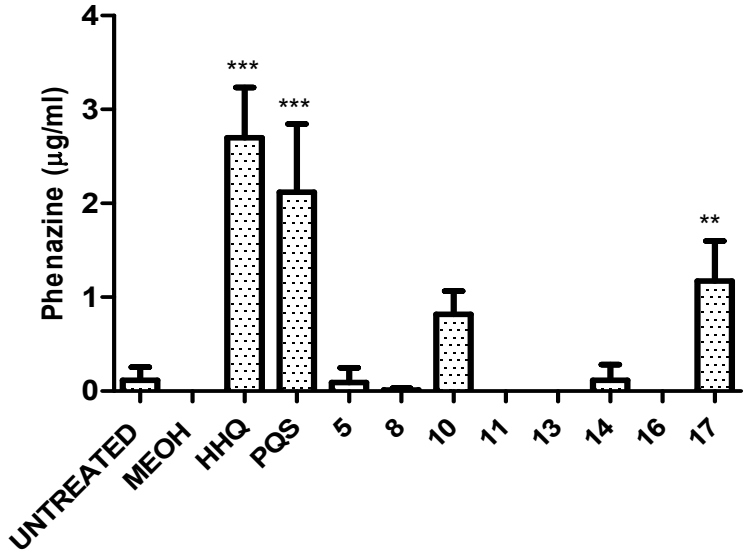
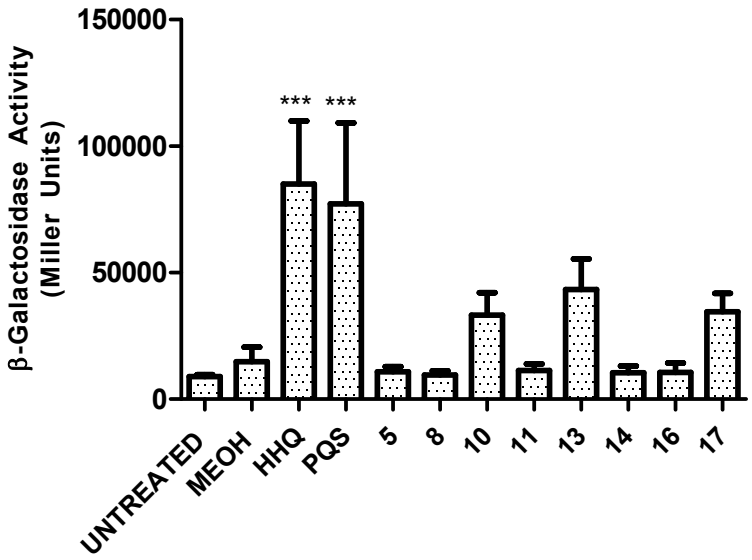

LDH Release
Percentage Cytotoxicity
80
60
40
20
0
*
***
***
MEOH
HHQ
PQS
5
8
10
11
13
14
16
17
100 µM – 16 hr

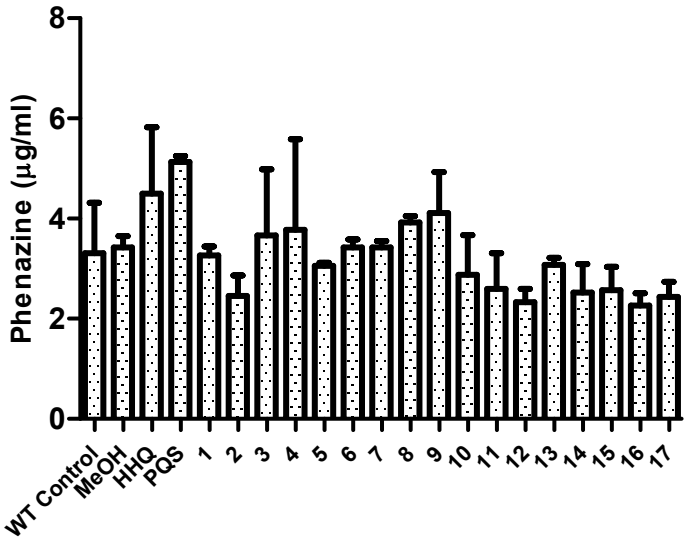
Figure S4

(a)


Phenazine Production in PAO1 pqsA- mutant
Phenazine (µg/ml)
0
1
2
3
4
***
***
**
UNTREATED
MEOH
HHQ
PQS
5
8
10
11
13
14
16
17


pqsA-lacZ Promoter Fusion Analysis in PAO1 pqsA- mutant
β-Galactosidase Activity (Miller Units)
0
50000
100000
150000
*** ***
UNTREATED
MEOH
HHQ
PQS
5
8
10
11
13
14
16
17

(b)


Phenazine Production in PAO1
Phenazine (µg/ml)
0
2
4
6
8
WT Control
MeOH
HHQ
PQS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

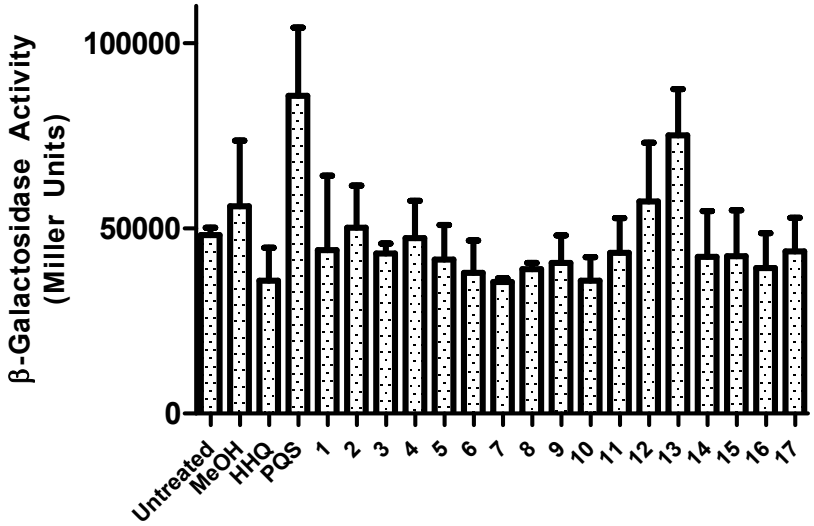

pqsA-lacZ Promoter Fusion Analysis in PAO1
β-Galactosidase Activity (Miller Units)
0
50000
100000
Untreated
MeOH
HHQ
PQS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

Figure S5

Figure S1. Lead compounds target biofilm but not planktonic related growth of *C. albicans*. (a) Viable cell counts from biofilms formed in the presence of selected lead compounds were recorded. Data shown is the average of four independent biological replicates. **(b)** Growth curve analysis of *C. albicans* SC5314 planktonic cells in multi-well plates. Data presented is the average of three independent biological replicates. Statistical analysis on both datasets was performed by one-way ANOVA with Bonferroni corrective testing (* $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$).

Figure S2. Microscopic analysis of inactive analogues. (a) Analogues **5**, **11**, **13**, **14** and **16** do not have any effect on *C. albicans* biofilm structure. Filamentous *C. albicans* biofilm in the presence of analogues (100 μM) was stained for chitin and cellulose (calcofluor; blue), lectins which binds to sugars, glycolipids and glycoproteins (concanavalin A; green) and live-dead cells (FUN-1; red). *C. albicans* biofilm in the presence of analogues **5**, **11**, **13**, **14** and **16** grew with dense branched hyphae, intertwining mycelial structures surrounded by a secreted polysaccharide matrix. **(b)** Analogues **8**, **10** and **17** have major effects on *C. albicans* biofilm structure. Filamentous *C. albicans* biofilm in the presence of analogues (100 μM) was stained for chitin and cellulose (calcofluor; blue), lectins which binds to sugars, glycolipids and glycoproteins (concanavalin A; green) and live-dead cells (FUN-1; red). *C. albicans* biofilm in the presence of analogues **8**, **10** and **17** exhibited severely compromised biofilm structures; stunted and less abundant hyphae surrounded by large cellular debris fields (hyphae, undifferentiated cells and pseudohyphae).

Figure S3. The expression transcripts of *C. albicans* biofilm/cell wall genes were not significantly increased in the presence of HHQ. Transcript expression analysis (RT-PCR) of

a panel of biofilm genes was assessed in *C. albicans* grown in YNB-NP (filamentous inducing media) in 100 μM HHQ for 6 hours at 37°C. Extracted RNA was isolated and reverse transcribed and subjected to RT-PCR using the primers in (**Supplemental Table S1**). All data was normalized to a housekeeper gene (*ACT1*). Data is presented as mean +/- SD and it represents three independent biological replicates; *p, ≤0.05. *BCR1*, *CPH1*, *DCK1*, *EFB1*, *ESS1*, *HGT2*, *RBT1*, and *TUP1* were not significantly affected when compared to methanol treated cells.

Figure S4. Analogues that do not impact on *C. albicans* biofilm formation exhibit variable cytotoxic activity towards IB3-1 lung epithelial cells. Analogues identified in the XTT assay as impacting on *C. albicans* biofilm formation were assessed for cytotoxicity towards IB3-1 cells. Cytotoxicity is measured as a percentage of total lactate dehydrogenase (LDH) released from cells treated with 0.1% Triton X-100 (100% cytotoxicity). Data (means ± SEM) are representative of three independent biological experiments.

Figure S5. (a) Inactive non-agonistic analogues. Analogues that do not impact on *C. albicans* biofilm formation do not induce PQS-dependent virulence phenotypes in *P. aeruginosa*. Phenazine release is a hallmark virulence characteristic of *P. aeruginosa*. In a PAO1 *pqsA*$^{-}$ mutant, phenazine production was partially restored by analogues **12** and **17**. In the PAO1 *pqsA*$^{-}$ mutant strain carrying a *pqsA-lacZ* promoter fusion, analogues did not interfere with the restoration of *pqsA* promoter activity by PQS, with the exception of a partial inhibition of restoration in the presence of **12**, **15** and **17**. **(b)** Inactive analogues are not antagonistic towards PQS auto-induction in a wild-type PAOI strain. Analogues had no effect on phenazine production in the wild-type strain. Exogenously added analogues also did not affect *pqsA*

promoter activity in the wild-type strains. Data presented as mean +/- SEM is representative of at least three independent biological replicates.