

Cholesterol-terminated cationic lipidated oligomers (CLOs) as a new class of antifungals

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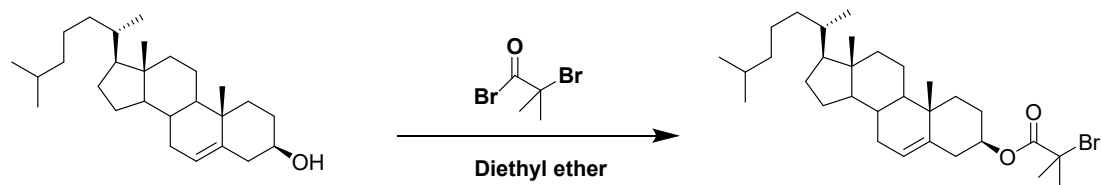
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Supplementary Information



SI Scheme 1: Synthesis of Cholesterol Initiator (Chol-Br).

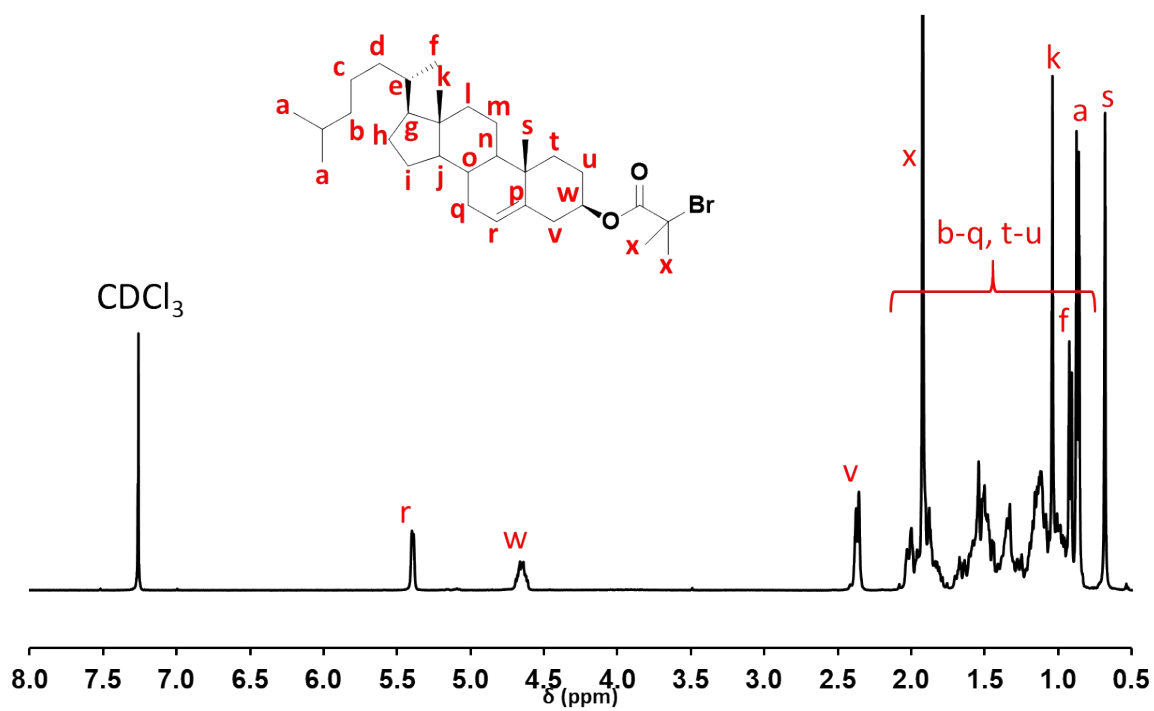


Figure SI 1: ^1H NMR spectra of Cholesterol Initiator (Chol-Br). ^1H NMR was conducted in CDCl_3 .

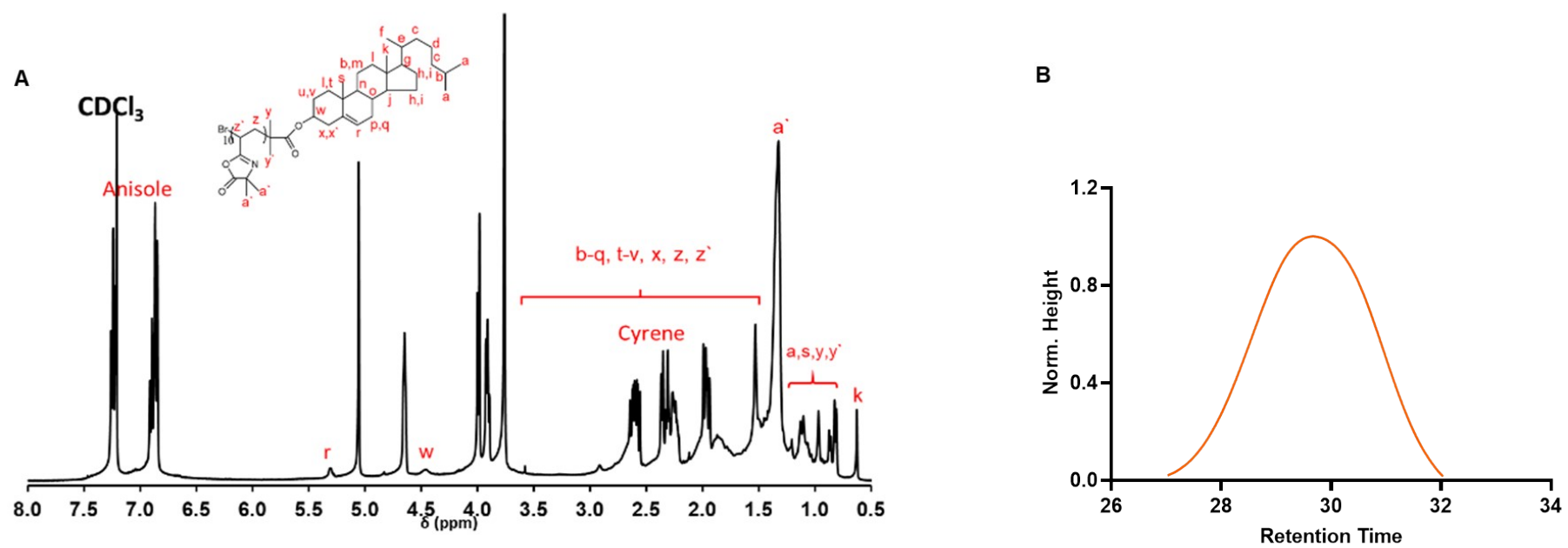


Figure SI 2: (A) ^1H NMR spectra of precursor oligomer Chol-VDM. ^1H NMR was conducted in CDCl_3 . (B) Molecular weight distribution over the time (min) for Cholesterol oligomers (DP-10).

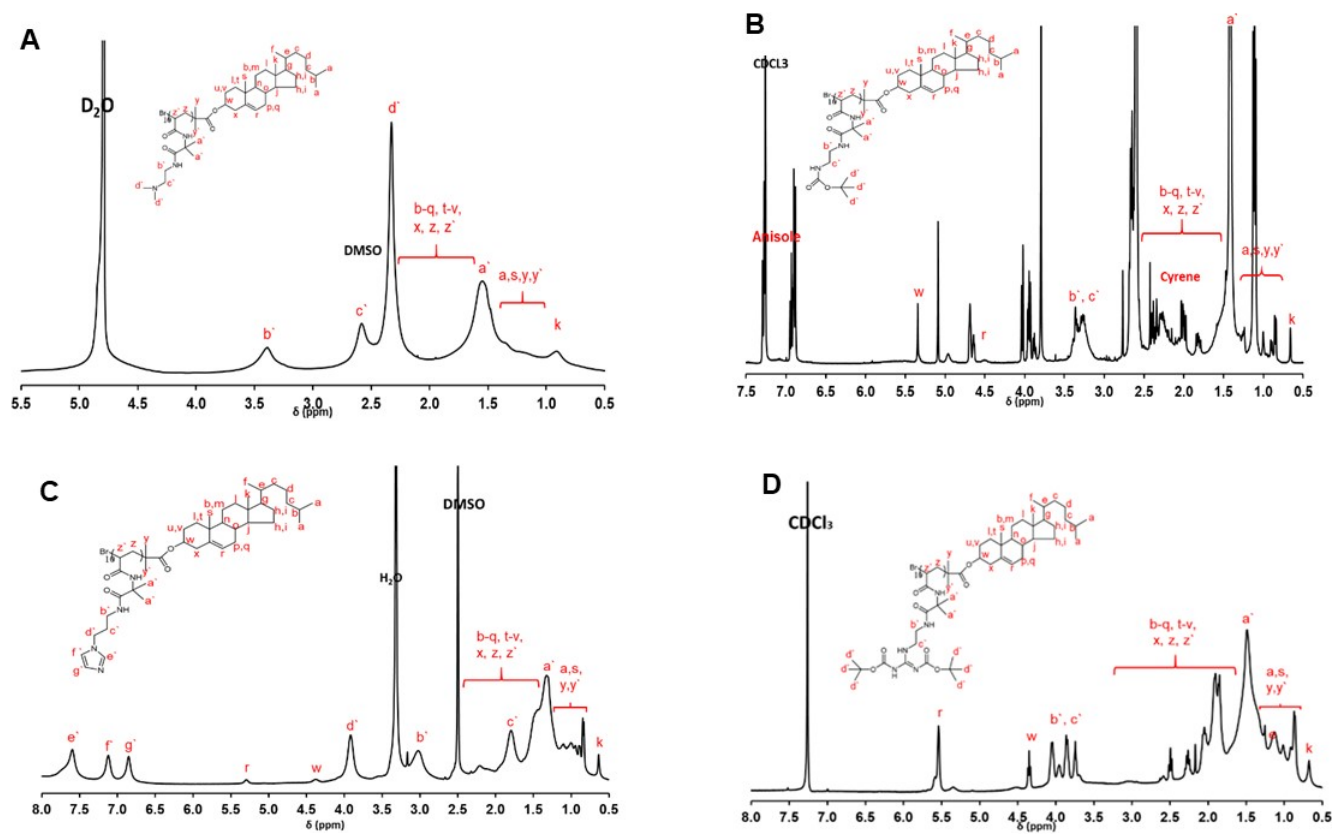


Figure SI 3: ^1H NMR spectra of Chol-VDM ring-opened utilizing A) *N,N*-dimethylethylenediamine (DMEN); B) *N*-Boc-ethylenediamine (BEDA); C) 1-(3-aminopropyl) imidazole (IMID); D) 2-(2-aminoethyl)-1,3-di-Boc-guanidine (BG). ^1H NMR spectra were recorded in D_2O for A, CDCl_3 for B and D, and DMSO-d_6 for C.

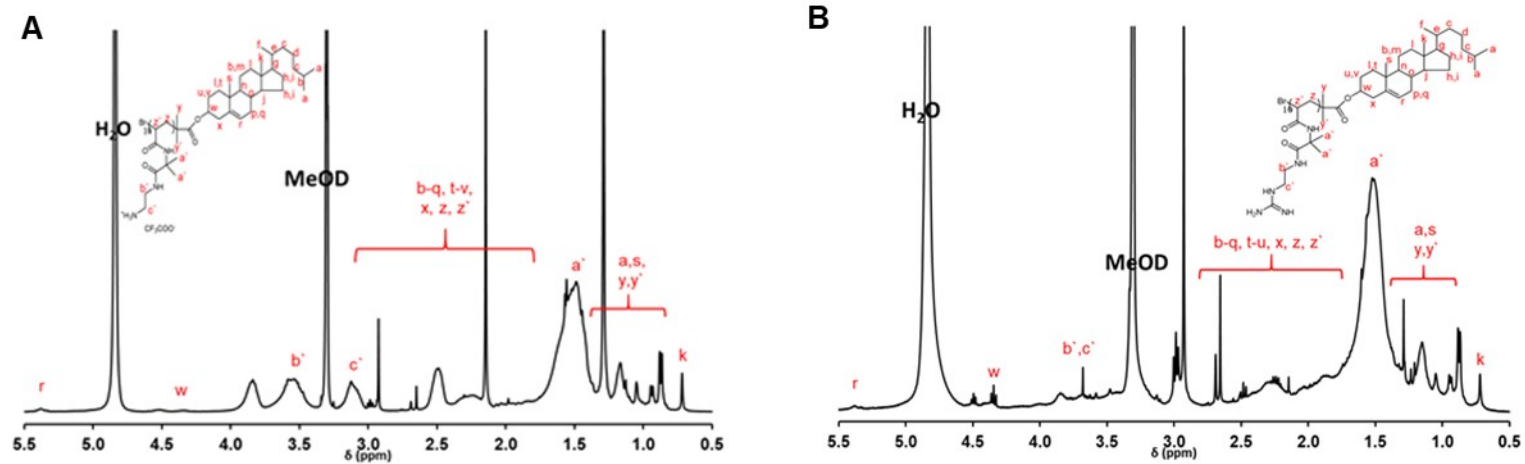


Figure SI 4: ^1H NMR spectra of deprotected oligomers; A) Chol-VDM(BEDA)-D; B) Chol-VDM(BG)-D. ^1H NMR spectra were recorded in MeOD.

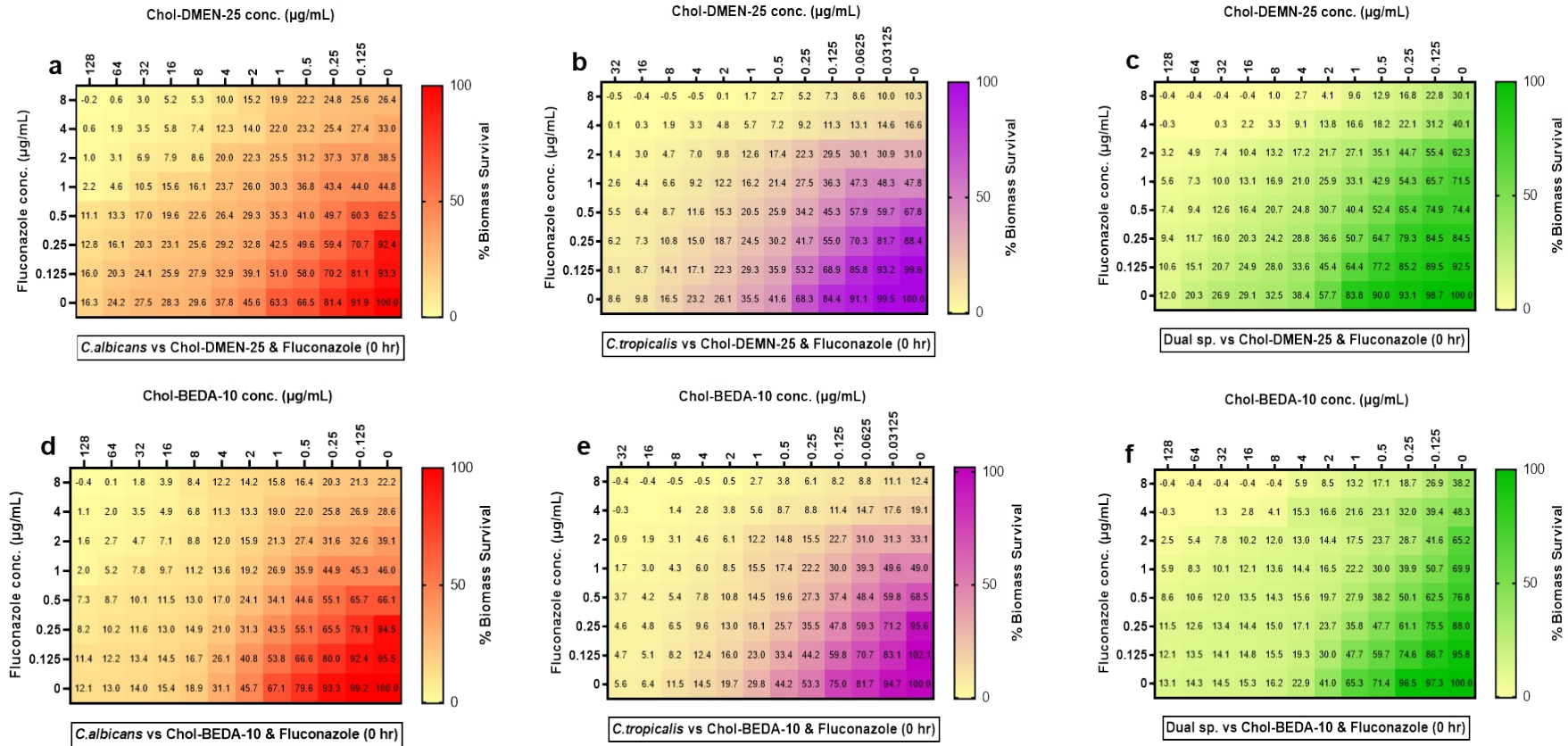


Figure SI 5: Checkerboards depicting fungal (*C. albicans*, *C. tropicalis*, and dual sp.) % biomass survival. a) Chol-DMEN-25(128 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) vs Fluconazole (8 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) against *C. albicans* planktonic cells. b) Chol-DMEN-25(32 $\mu\text{g/mL}$ - 0.031 $\mu\text{g/mL}$) vs Fluconazole (8 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) against *C. tropicalis* planktonic cells. c) Chol-DMEN-25(32 $\mu\text{g/mL}$ - 0.031 $\mu\text{g/mL}$) vs Fluconazole (8 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) against dual sp. planktonic cells. d) Chol-BEDA-10 (128 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) vs Fluconazole (8 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) against *C. albicans* planktonic cells. e) Chol-BEDA-10 (32 $\mu\text{g/mL}$ - 0.031 $\mu\text{g/mL}$) vs Fluconazole (8 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) against *C. tropicalis* planktonic cells. f) Chol-BEDA-10 (128 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) vs Fluconazole (8 $\mu\text{g/mL}$ - 0.125 $\mu\text{g/mL}$) against dual sp. planktonic cells.

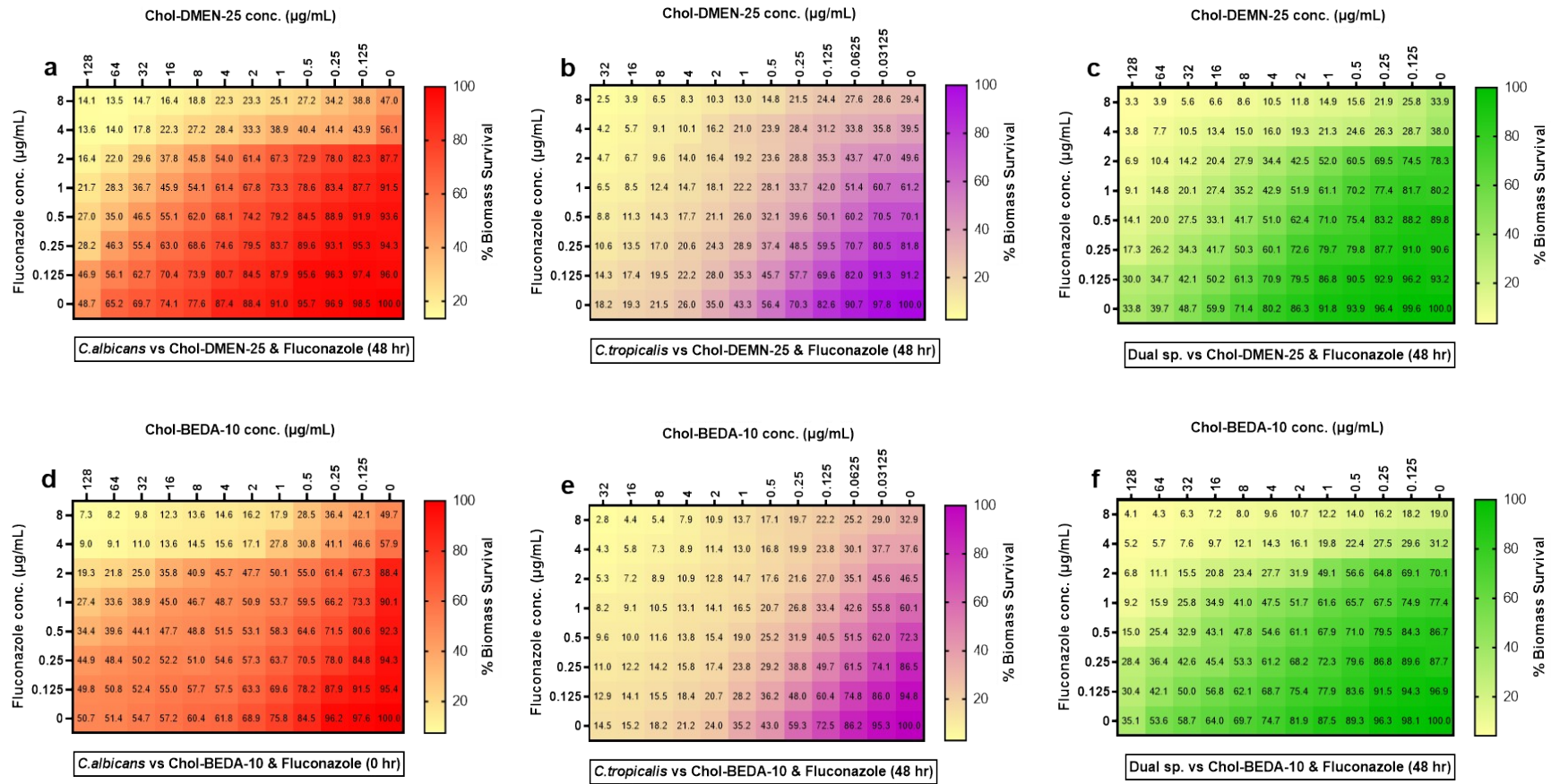


Figure SI 6: Checkerboards depicting fungal (*C. albicans*, *C. tropicalis*, and dual sp.) mature biofilm % biomass survival. a) Chol-DMEN-25(128 µg/mL - 0.125 µg/mL) vs Fluconazole (8 µg/mL - 0.125 µg/mL) against *C. albicans* biofilm. b) Chol-DMEN-25 (32 µg/mL - 0.031 µg/mL) vs Fluconazole (8 µg/mL - 0.125 µg/mL) against *C. tropicalis* biofilm. c) Chol-DMEN-25(32 µg/mL - 0.031 µg/mL) vs Fluconazole (8 µg/mL - 0.125 µg/mL) against dual sp. biofilm. d) Chol-BEDA-10 (128 µg/mL - 0.125 µg/mL) vs Fluconazole (8 µg/mL - 0.125 µg/mL) against *C. albicans* biofilm. e) Chol-BEDA-10 (32 µg/mL - 0.031 µg/mL) vs Fluconazole (8 µg/mL - 0.125 µg/mL) against *C. tropicalis* biofilm. f) Chol-BEDA-10 (128 µg/mL - 0.125 µg/mL) vs Fluconazole (8 µg/mL - 0.125 µg/mL) against dual sp. biofilm.

Table SI 1: Solvents used for NMR

CLOs Series	¹H NMR Solvents		
	Synthesis	Ring Opening	Deprotection
Chol-OVDM(DMEN)	CDCl ₃	D ₂ O	-
Chol-OVDM(BEDA)	CDCl ₃	CDCl ₃	CH ₃ OD
Chol-OVDM(IMID)	CDCl ₃	DMSO-d ₆	-
Chol-OVDM(BG)	CDCl ₃	CDCl ₃	CH ₃ OD

Table SI 2: Solvents used for dialysis

CLOs Series	Dialysis Solvents
Chol-OVDM(DMEN)	Deionized water
Chol-OVDM(BEDA)	Acetone
Chol-OVDM(IMID)	Methanol
Chol-OVDM(BG)	Acetone

Table SI 3: Antibacterial and toxicity profile of CLOs against *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *A. baumannii* ATCC 19606, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 43300 MRSA.

CLOs	MIC					Cytotoxicity ($\mu\text{g/mL}$)	Haemolysis	
	($\mu\text{g/mL}$)						($\mu\text{g/mL}$)	
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	CC ₅₀	HC ₁₀	HC ₅₀
Chol-OVDM(DMEN)-5	>640	>640	320	>640	640	32-126*	4	3-9
Chol-OVDM(DMEN)-10	>640	>640	160	>640	>640	8-86	1	3
Chol-OVDM(DMEN)-15	>640	>640	320	>640	>640	3-103	1	3-5
Chol-OVDM(DMEN)-20	>640	>640	>640	>640	>640	38-109	1 to 2	5-9
Chol-OVDM(DMEN)-25	>640	>640	>640	>640	>640	5->128*	2 to 3	9-33
Chol-OVDM(BEDA)-5	>640	>640	>640	>640	>640	53->128	5	8-15
Chol-OVDM(BEDA)-10	>640	>640	>640	>640	320	8->128*	3	8->128*
Chol-OVDM(IMID)-5	>640	>640	>640	>640	>640	35->128*	1 to 3	126->128
Chol-OVDM(IMID)-10	>640	>640	>640	>640	>640	>128	<1	>128
Chol-OVDM(BG)-5	>640	>640	>640	>640	>640	>128	79-96	>128
Chol-OVDM(BG)-10	>640	>640	>640	>640	>640	125->128	86->128	31-120*
Vancomycin					0.25			
Tamoxifen						5 to 29		
Melittin							1 to 13	8

Table SI 4: Selectivity indices for the CLOs against *C. tropicalis*, *C. glabrata*, *C. deuterigattii* and *C. auris*. The selectivity index is calculated by dividing the HC₁₀ by the MIC value for a given microbe.

CLOs	HC ₁₀ (µg/mL)	Selectivity index value			
		<i>C. tropicalis</i>	<i>C. glabrata</i>	<i>C. deuterigattii</i>	<i>C. auris</i>
Chol-OVDM(DMEN)-5	4	0.4-3.2	0.05	0.0062-0.2	0.0062-0.8
Chol-OVDM(DMEN)-10	1	0.8-1.58	0.025-0.05	0.2-0.4	0.0015-12.5
Chol-OVDM(DMEN)-15	1	0.8-1.58	0.0125-0.025	0.2-0.4	0.025-0.4
Chol-OVDM(DMEN)-20	1-2	1.58-1.6	0.00625	0.1-0.2	0.0031-0.1
Chol-OVDM(DMEN)-25	2-3	2.4-3.1	0.0093-0.0125	0.3-0.4	0.004-0.4
Chol-OVDM(BEDA)-5	5	0.25-0.5	0.0078-0.015	0.007-.125	0.007-2
Chol-OVDM(BEDA)-10	3	1.2-2.4	0.018	0.25-0.3	0.004-0.6
Chol-OVDM(IMID)-5	1-3	0.6-0.8	0.0093-0.025	0.004-0.1	0.004-0.2
Chol-OVDM(IMID)-10	<1	0.8-3.2	0.0062-0.05	0.05-0.2	0.0015-0.2
Chol-OVDM(BG)-5	79-96	0.15-1.9	0.15-7.9	0.1-0.12	0.15-3.95

Chol-OVDM(BG)-10	86≥128	0.8-17.2	0.13-0.2	0.13-0.2	0.2-17.2
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Table SI 5: Selectivity indices for the oligomer library against *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *S. aureus*. The selectivity index is calculated by dividing the HC₁₀ by the MIC value for a given microbe.

CLOs	HC ₁₀ (µg/mL)	Selectivity index value				
		<i>E. coli</i>	<i>K. pneumoniae</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Chol-OVDM(DMEN)-5	4	>0.006	>0.006	0.013	>0.006	0.006
Chol-OVDM(DMEN)-10	1	>0.002	>0.002	0.006	>0.002	>0.002
Chol-OVDM(DMEN)-15	1	>0.002	>0.002	0.003	>0.002	>0.002
Chol-OVDM(DMEN)-20	1-2	>0.001-0.003	>0.001-0.003	>0.001-0.003	>0.001-0.003	>0.001-0.003
Chol-OVDM(DMEN)-25	2-3	>0.003-0.004	>0.003-0.004	>0.003-0.004	>0.003-0.004	>0.003-0.004
Chol-OVDM(BEDA)-5	5	>0.008	>0.008	>0.008	>0.008	>0.008
Chol-OVDM(BEDA)-10	3	>0.005	>0.005	>0.005	>0.005	0.009
Chol-OVDM(IMID)-5	1-3	>0.001-0.004	>0.001-0.004	>0.001-0.004	>0.001-0.004	>0.001-0.004
Chol-OVDM(IMID)-10	<1	>0.002	>0.002	>0.002	>0.002	>0.002
Chol-OVDM(BG)-5	79-96	>0.12-0.15	>0.12-0.15	>0.12-0.15	>0.12-0.15	>0.12-0.15
Chol-OVDM(BG)-10	86≥128	>0.13-0.2	>0.13-0.2	>0.13-0.2	>0.13-0.2	>0.13-0.2