Supplementary Information

Title: Organoselenium has potent fungicidal effect on *Cryptococcus neoformans* and inhibits the virulence factors

Running title: Organoselenium: a fungicidal and anti-virulence compound

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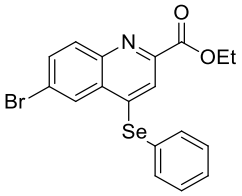
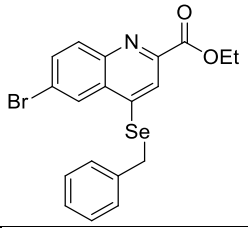
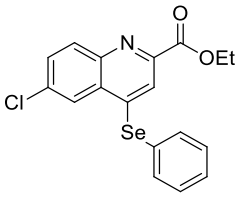
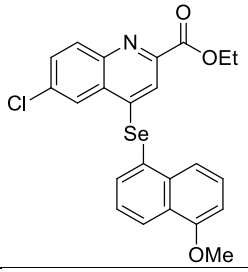
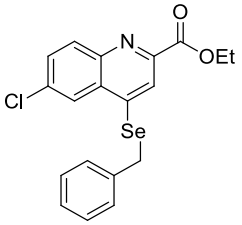
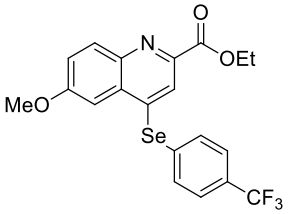
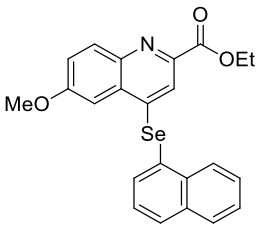
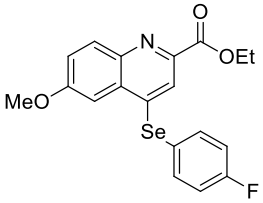
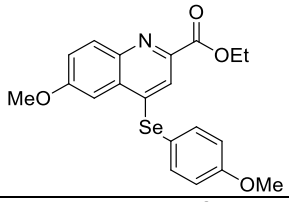
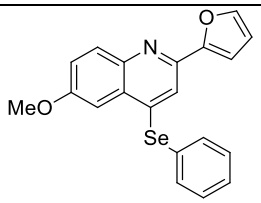
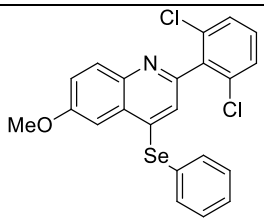
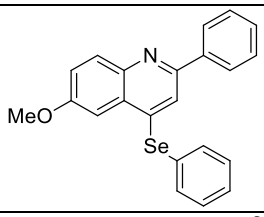
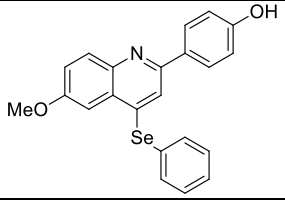
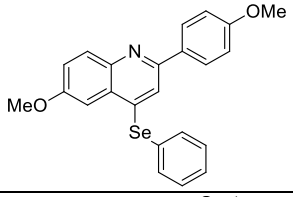
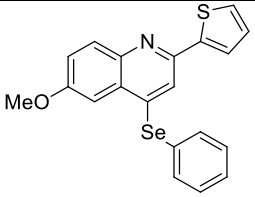
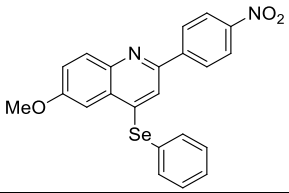
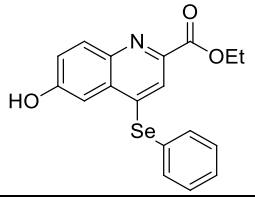
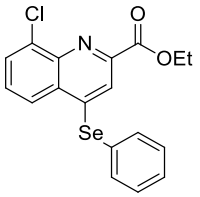
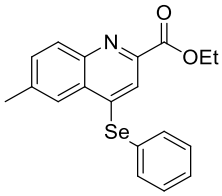
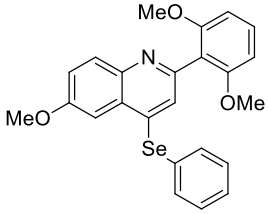
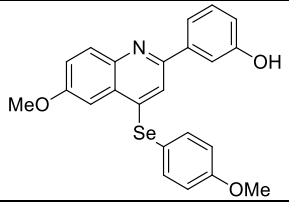
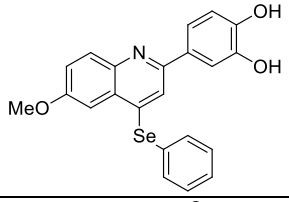
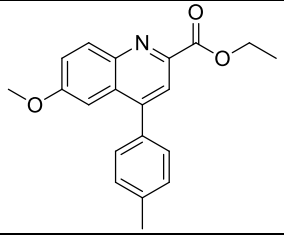
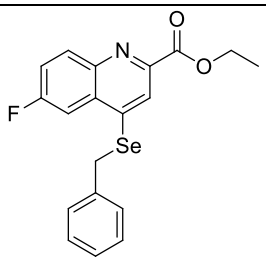
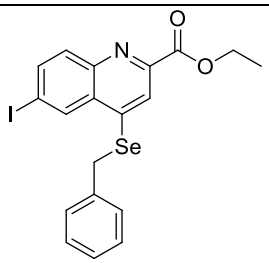
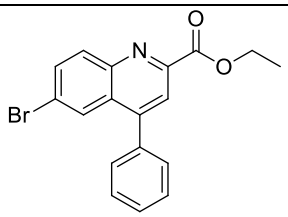
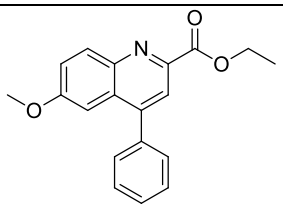
Table S1. Antifungal activity of synthetic compounds against *Aspergillus fumigatus* ATCC 16913, *Candida albicans* SC5314 and *Candida neoformans* H99 and hemolytic activity on red blood cells.

Compound	Molecular structure	IC_{50}/IC_{90}/MFC (µg/mL)			HA_{50} (µg/mL)
		Cryptococcus neoformans	*Candida albicans*	*Aspergillus fumigattus*	
1		>128	>128	>128	nd
2		4/8/16	8/16/32	>128	>128
3		>128	>128	>128	nd
4		>128	>128	>128	nd
5		4/8/16	64/>128/>128	>128	>128
6		>128	>128	>128	nd

7		>128	>128	>128	nd
8		>128	>128	>128	nd
9		16/64/128	64/>128/>128	>128	>128
10		>128	>128	>128	nd
11		>128	>128	>128	nd
12		32/64/>128	>128	>128	nd
13		64/128/>128	>128	>128	nd
14		16/128/>128	>128	>128	>128
15		>128	>128	>128	nd

16		16/32/>128	>128	>128	>128
17		>128	>128	>128	nd
18		>128	>128	>128	nd
19		>128	>128	>128	nd
20		64/>128/>128	>128	>128	nd
21		>128	>128	>128	nd
22		4/8/>128	16/>128/>128	128	>128
23		>128	>128	>128	nd

24		8/32/32	>128	>128	>128
25		8/16/32	>128	>128	>128
26		>128	>128	>128	nd
27		>128	>128	>128	nd

IC_{50}, the lowest concentration that inhibits 50% of fungal growth

IC_{90}, the lowest concentration that inhibits 90% of fungal growth

MFC, the lowest concentration that kills >99.9% of yeasts

HA_{50}, the lowest concentration that leads to 50 % hemolysis of red blood cells

nd, not determined

Table S2. Susceptibility of *Cryptococcus neoformans* strains to the organoselenium compound LQA_78 and the standard antifungals fluconazole (FLC) and amphotericin B (AMB). The concentration values are expressed in μg/mL.

Strains	AMB			FLC			LQA_78		
	IC_{50}	IC_{90}	MFC	IC_{50}	IC_{90}	MFC	IC_{50}	IC_{90}	MFC
H99 NA	0.016	0.03	0.06	1	2	8	4	8	16
H99 A	0.016	0.03	0.06	4	8	64	4	8	16
CAP59	0.03	0.03	0.03	0.5	1	8	2	4	8
L53	0.06	0.25	0.25	1	2	4	8	16	32
L177	0.06	0.125	0.25	2	4	8	8	16	32
L331	0.06	0.125	0.25	1	2	4	8	16	32
L333	0.03	0.06	0.125	2	4	16	16	32	64
L354	0.25	0.25	0.5	4	8	16	8	16	32
L378	0.25	0.5	1	2	4	16	16	32	64
L449	0.06	0.125	0.25	2	4	16	16	32	64
L576	0.125	0.25	0.5	2	4	8	16	32	64
L580	0.125	0.25	0.5	2	4	8	16	32	64
541	0.03	0.03	0.03	0.25	0.25	0.25	1	2	8
542	0.03	0.03	0.125	4	8	64	1	2	4
543	0.125	0.25	1	0.25	0.25	2	1	4	4
544A	0.25	0.25	0.25	1	2	4	4	4	8
545A	0.125	0.25	0.5	1	2	8	4	8	8

IC_{50}, the lowest concentration that inhibits 50% of fungal growth

IC_{90}, the lowest concentration that inhibits 90% of fungal growth

MFC, the lowest concentration that kills >99.9% of cells

The assays were performed in duplicate at least three times. The data represent the modal mean.

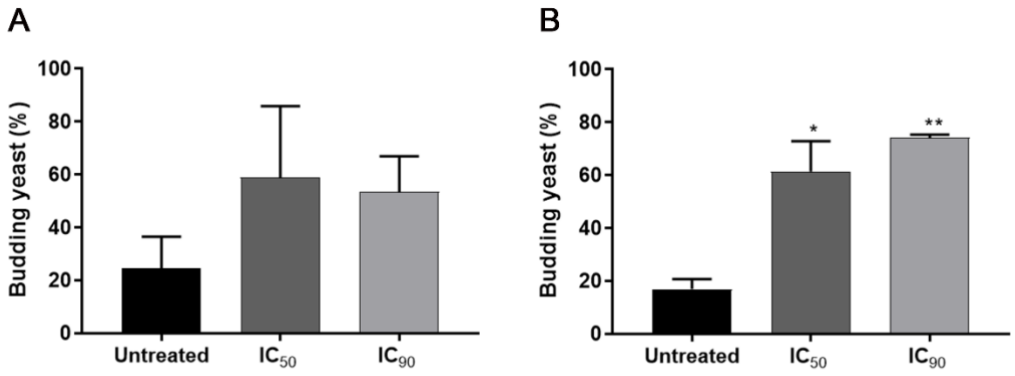


Figure S1. The compound LQA_78 inhibits *Cryptococcus neoformans* growth, resulting in budding yeast accumulation. **(A)** H99 adapted strain and **(B)** clinical isolate L354. *p<0.05 and **p<0.01 compared to the untreated group (one-way ANOVA with Dunnett's post-test). Two independent experiments were performed in duplicate.

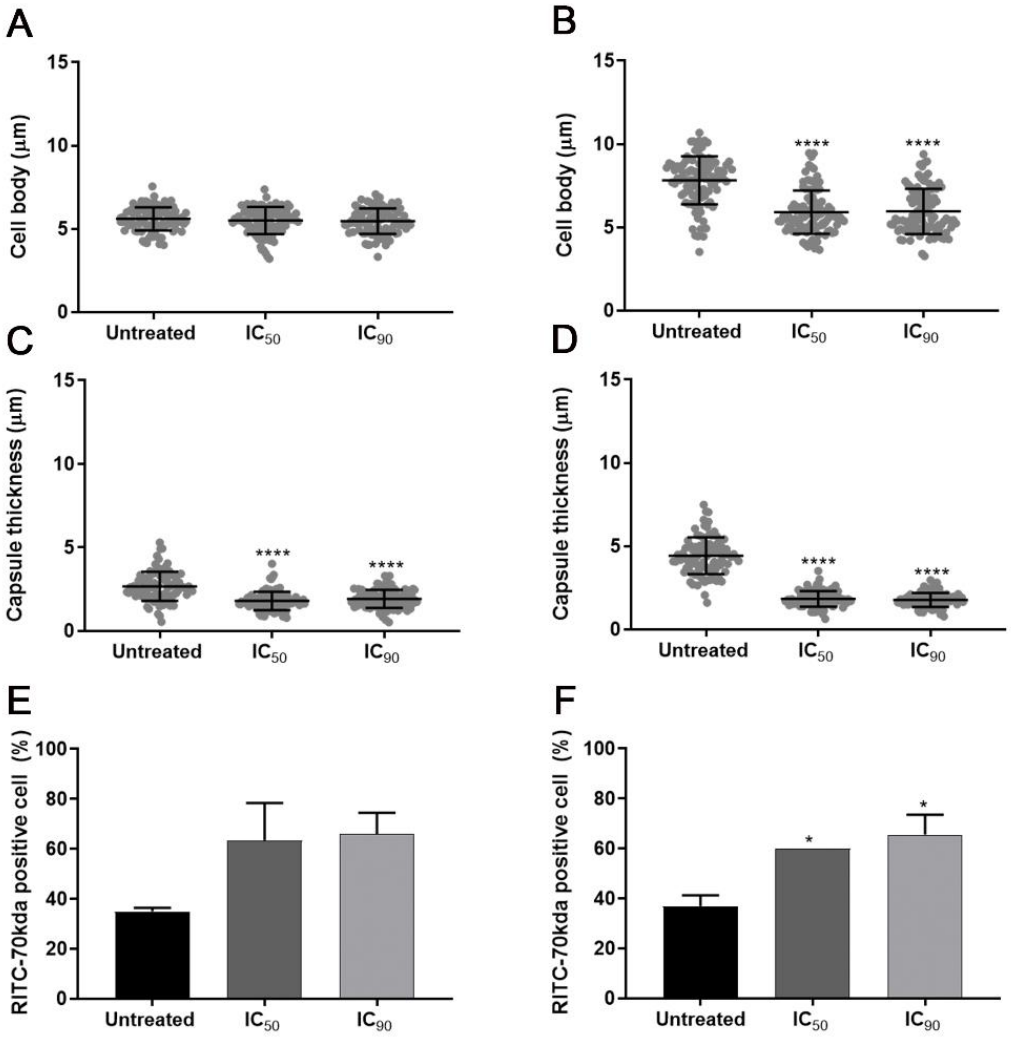


Figure S2. Inhibitory effect of LQA_78 on *Cryptococcus neoformans* H99 adapted strain (left column) and clinical isolate L354 (right column) virulence factors. **(A-B)** Cell diameter, **(C-D)** capsule thickness, **(E-F)** capsule permeability. *p<0.05 and ****p <0.0001 compared with the untreated group (one-way ANOVA with Dunnett's post-test). n=50-100 cells. Two independent experiments were performed in duplicate.

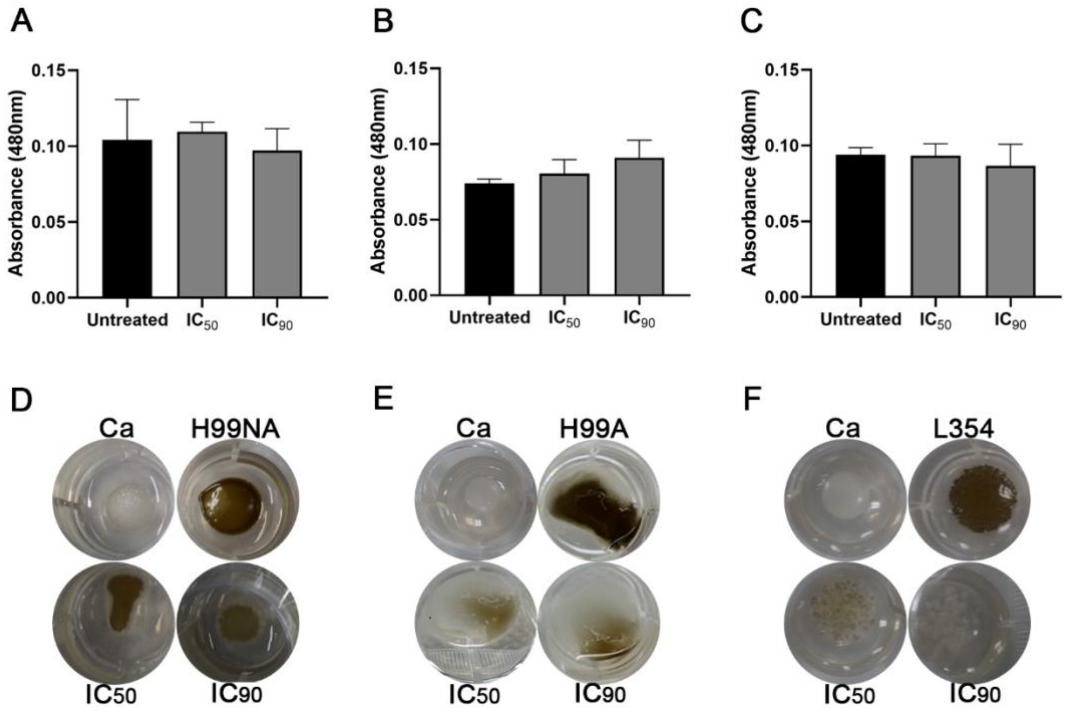


Figure S3. Inhibitory effect of LQA_78 on melanin leakage (A-C) and melanin production (D-F) of *Cryptococcus neoformans* strains after 72 h of incubation at 30°C. **(A, D)** H99 nonadapted strain, **(B, E)** H99 adapted strain, and **(C, F)** clinical isolate L354. Two independent experiments in duplicate (one-way ANOVA with Dunnett's post-test).

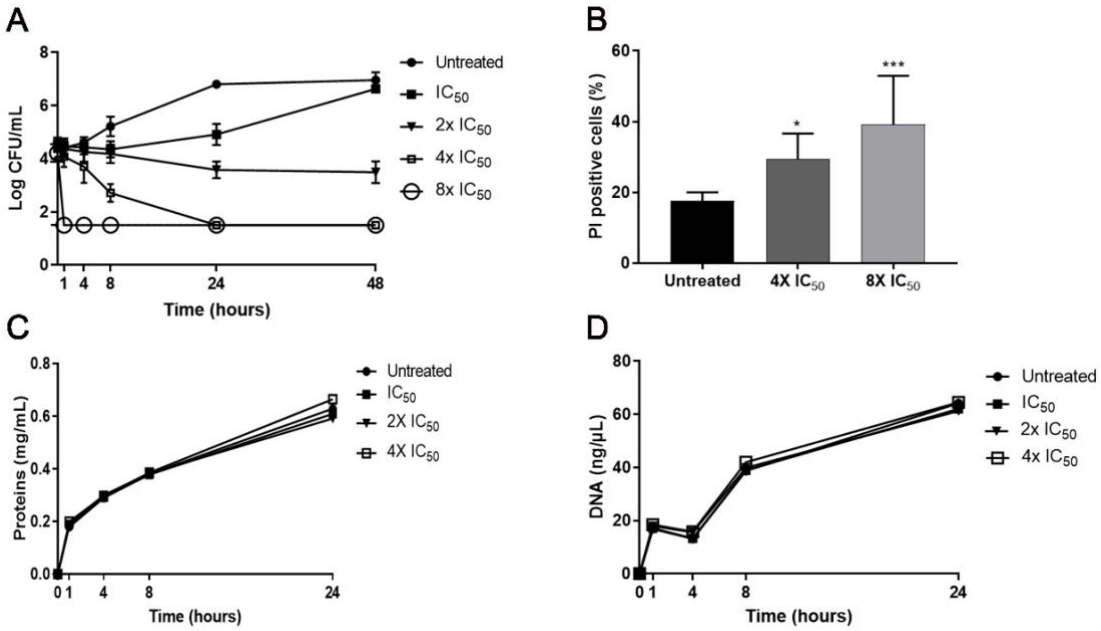


Figure S4. Fungicidal effect of LQA_78 on *Cryptococcus neoformans* H99 (adapted strain). **(A)** Time-kill curve, **(B)** flow cytometry analysis by propidium iodide (PI) after 24 h of incubation with LQA_78, **(C-D)** quantification of protein **(C)** and DNA **(D)** on supernatant after 0, 1, 4, 8 and 24 h of treatment. *p <0.05 and ***p <0.001 compared with the untreated group of three independent experiments [one-way ANOVA with Dunnett's post-test for (B) and two-way ANOVA for **(**C) and (D)].

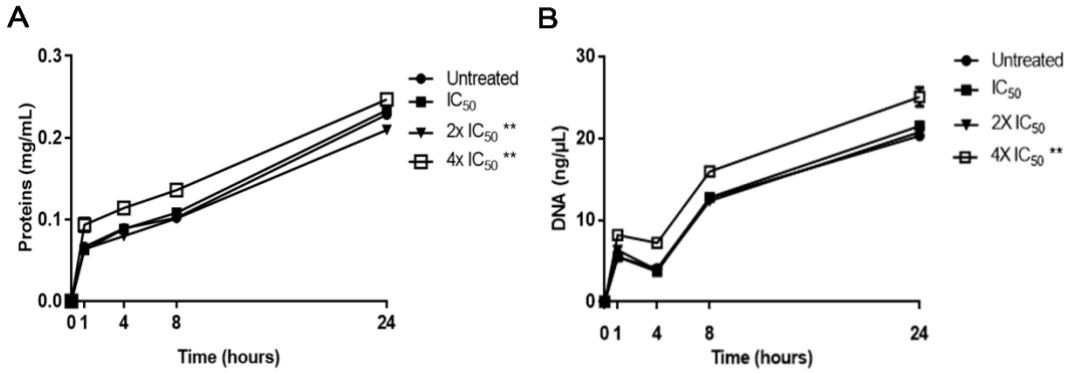


Figure S5. Cytoplasm leakage of *Cryptococcus neoformans* H99 (non-adapted strain) treated with LQA_78. **(A)** Quantification of protein and **(B)** DNA on supernatant after 0, 1, 4, 8 and 24 h of treatment. **p <0.01 compared with the untreated group of three independent experiments (two-way ANOVA with Dunnett's post-test).