Supporting Information

Celastrol inhibits *Plasmodium falciparum* enoyl-acyl carrier protein reductase

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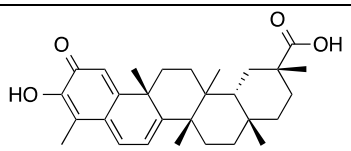
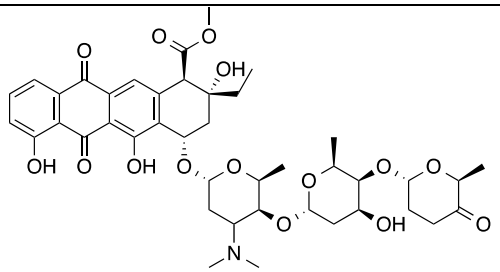
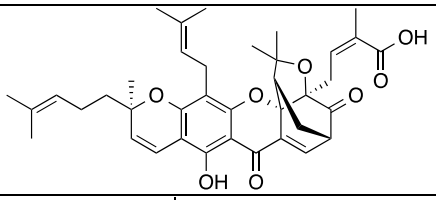
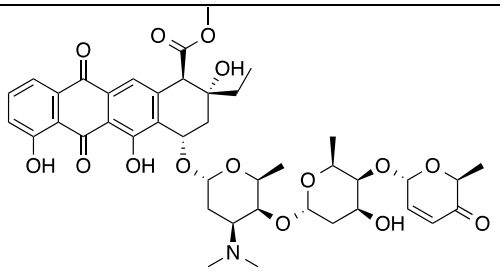
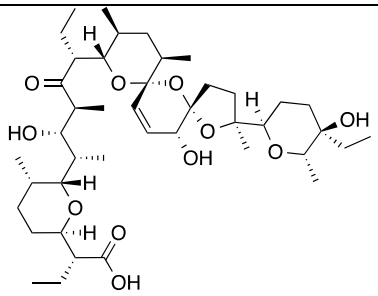
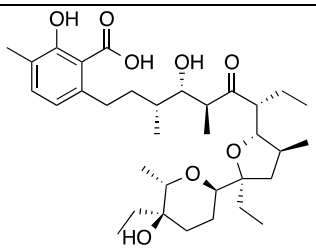
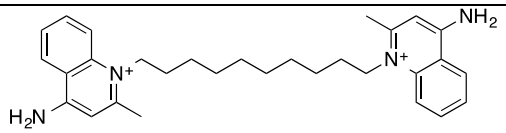
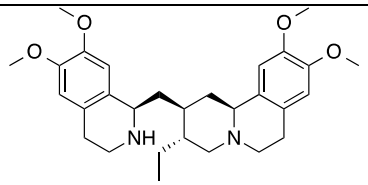
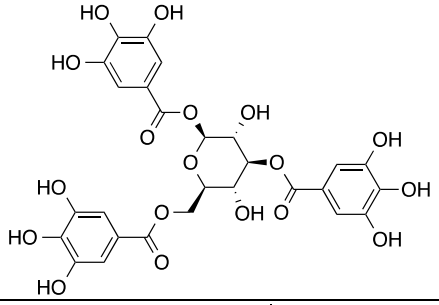
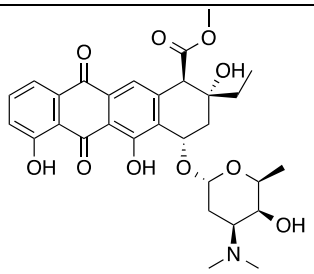
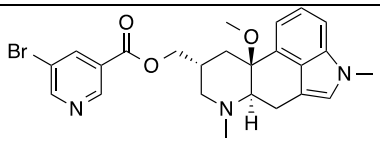
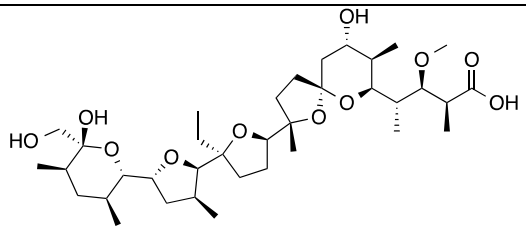
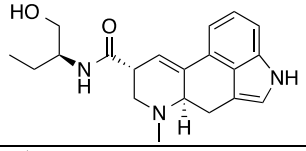
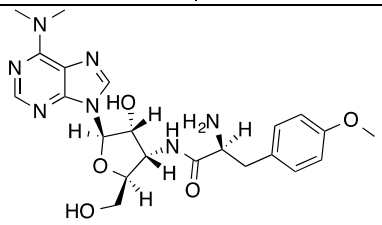
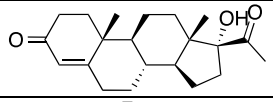
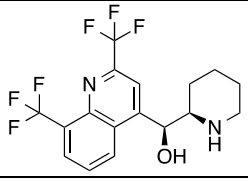
SI References

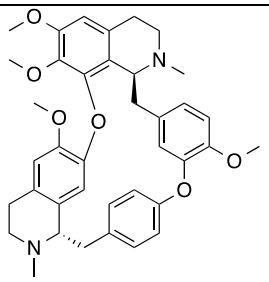
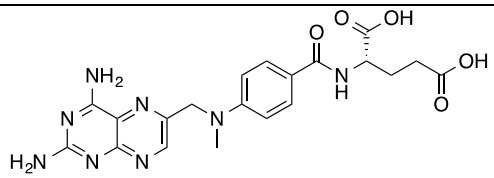
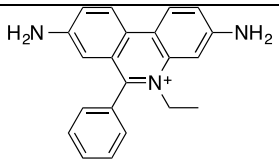
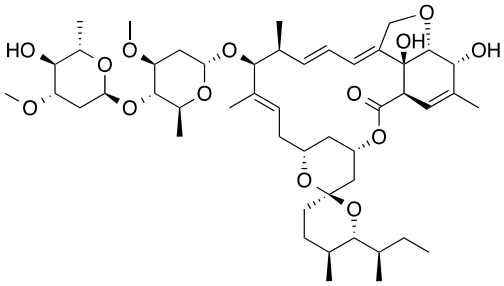
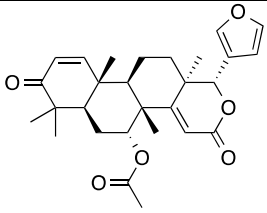
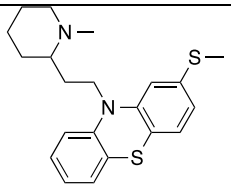
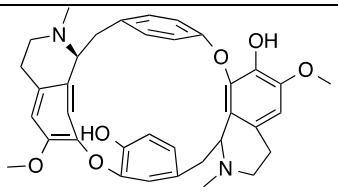
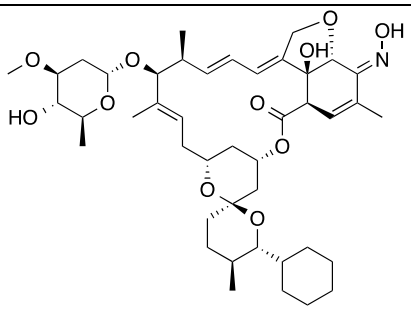
(1) Weisman JL, Liou AP, Shelat AA, Cohen FE, Kiplin Guy R, et al. (2006) Searching for New Antimalarial Therapeutics amongst Known Drugs. Chem Biol Drug Des 67: 409–416. doi:10.1111/j.1747-0285.2006.00391.

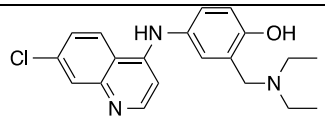
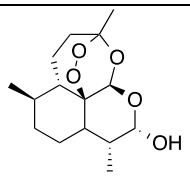
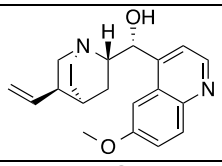
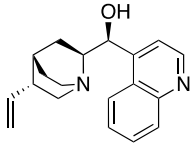
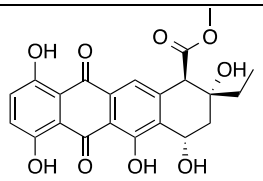
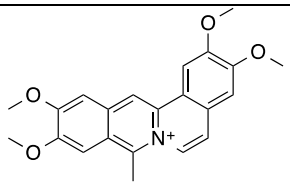
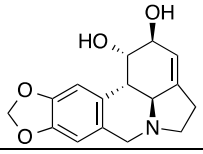
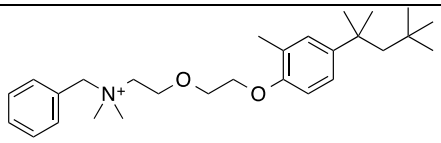
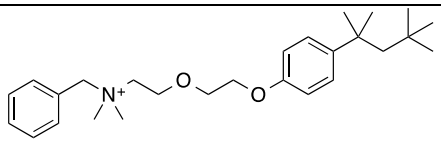
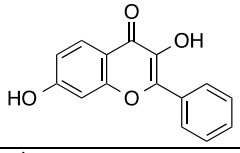
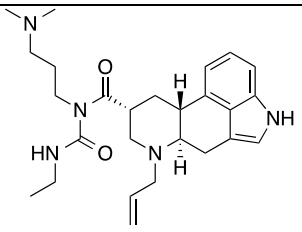
(2) Kapoor M, Jamal Dar M, Surolia A, Surolia N (2001) Kinetic Determinants of the Interaction of Enoyl-ACP Reductase from *Plasmodium falciparum* with Its Substrates and Inhibitors. Biochem Biophys Res Commun 289: 832–837. doi:10.1006/bbrc.2001.6061.

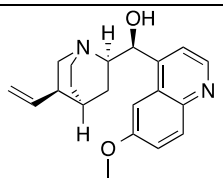
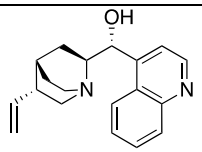
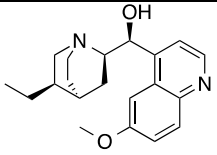
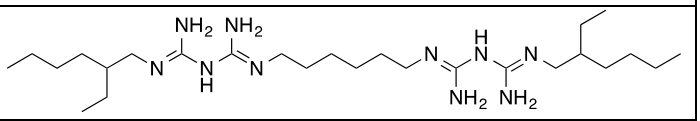
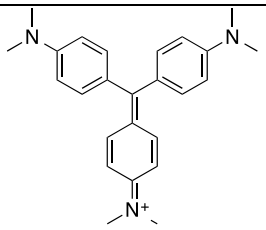
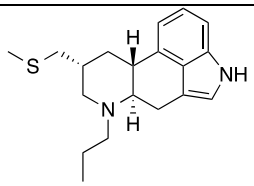
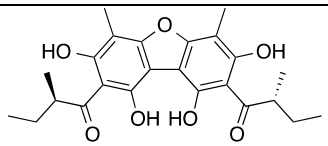
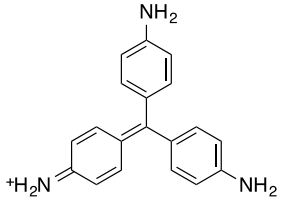
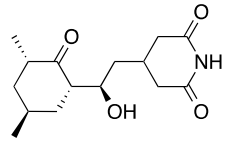
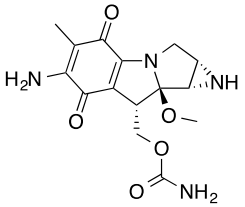
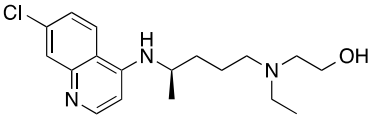
Table S1

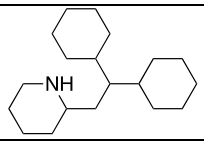
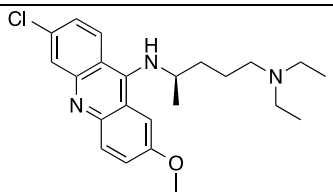
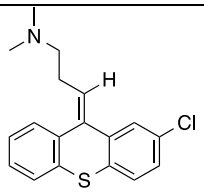
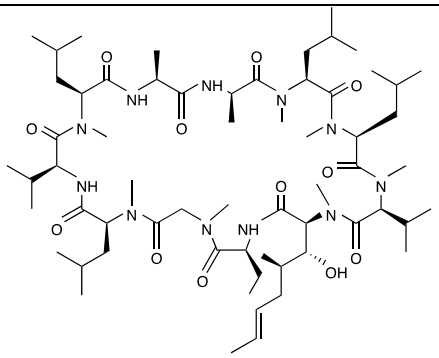
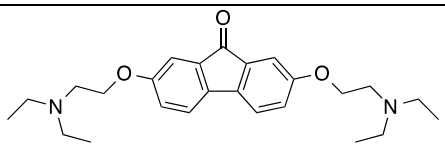
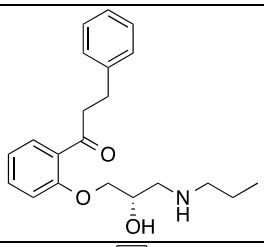
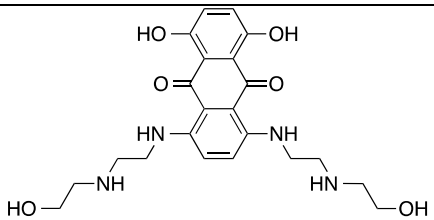
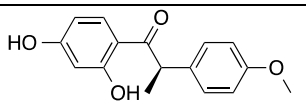
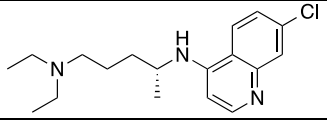
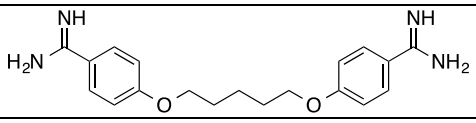
Name	PDB ID	Score (kcal/mol)	Structure
Celastrol	2OL4:A	-12.8	
Aclarubicin	1UH5:B	-12.6	
Gambogic acid	1ZSN:B	-12.6	
Aclacinomycin Y	2OL4:A	-12.3	
Salinomycin	1UH5:B	-11.9	
Lasalocid	2O2Y:D	-11.8	
Dequalinium	1VRW:B	-11.5	

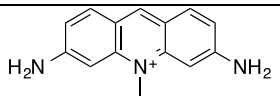
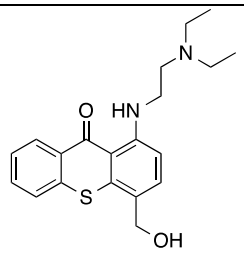
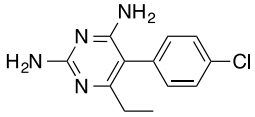
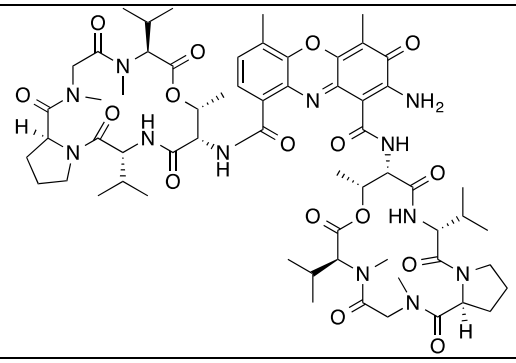
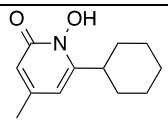
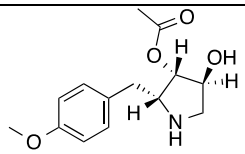
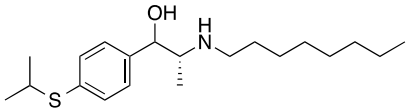
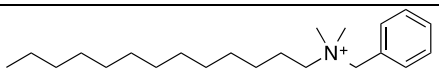
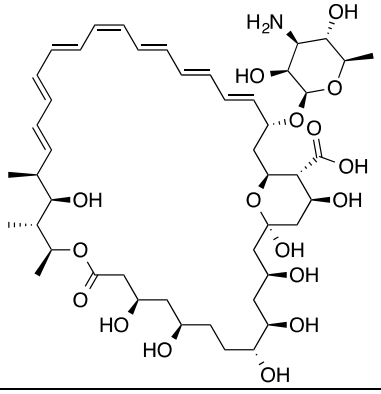
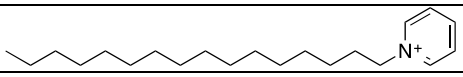
Emetine	2OOS:B	-11.5	
Tannic acid	1NNU:A	-11.4	
Aklavine hydrochloride	1V35:B	-11.2	
Nicergoline	1VRW:B	-11.2	
Monensin sodium	2NQ8:B	-11.2	
Methylergonovine	2O2Y:C	-11.1	
Puromycin	2OL4:B	-11.1	
Hydroxyprogesterone	2OOS:B	-10.7	
Mefloquine	1VRW:A	-10.6	

Tetrandrine	2OL4:A	-10.6	
Methotrexate	2OL4:B	-10.6	
Homidium	2OL4:B	-10.4	
Ivermectin	2OL4:A	-10.2	
Deoxygeduniun	1VRW:A	-10.1	
Thioridazine	2OP0:B	-10.1	
Bebeerine	1NNU:A	-10.0	
Selamectin	2NQ8:B	-10.0	

Amodiaquine	2OOS:B	-10.0	
Dihydroartemisinin	1ZW1:A	-9.9	
Quinine	2OL4:B	-9.9	
Cinchonine	2OL4:B	-9.9	
Rutilantinone	1V35:A	-9.8	
Coralyne	1VRW:A	-9.8	
Lycorine	1VRW:B	-9.8	
Methylbenzethonium	1VRW:B	-9.8	
Benzethonium	2O2Y:C	-9.8	
3,7-Dihydroxyflavone	2OOS:B	-9.8	
Cabergoline	2OOS:B	-9.8	

Quinidine	1VRW:A	-9.7	
Cinchonidine	2OL4:B	-9.7	
Hydroquinidine	2OL4:B	-9.7	
Alexidine hydrochloride	2OP0:B	-9.7	
Gentian violet	1ZXB:B	-9.5	
Pergolide	1VRW:B	-9.4	
Rhodomyrtoxin B	1ZXB:A	-9.4	
Pararosaniline	1UH5:A	-9.3	
Cycloheximide	1VRW:B	-9.3	
Mitomycin	1ZXB:A	-9.3	
Hydroxychloroquine	2OOS:B	-9.3	

Perhexiline	2OP1:B	-9.1	
Quinacrine	1UH5:A	-9.0	
Chlorprothixene	1VRW:A	-9.0	
Cyclosporin A	2OL4:A	-9.0	
Tilorone	2OL4:B	-9.0	
Propafenone	1UH5:B	-8.9	
Mitoxantrone	1VRW:B	-8.9	
Angloensin	2O2Y:C	-8.8	
Chloroquine	2OOS:B	-8.8	
Pentamidine	2OOS:B	-8.8	

Acriflavinium	1ZW1:A	-8.7	
Hycanthone	2OP0:B	-8.7	
Pyrimethamine	1NNU:B	-8.4	
Dactinomycin	2OL4:A	-8.4	
Ciclopirox	1NNU:B	-8.3	
Anisomycin	2OOS:B	-8.2	
Sulcotidil	1VRW:B	-7.6	
Benzalkonium	2OL4:B	-7.3	
Amphotericin B	2OP1:A	-7.1	
Cetylpyridinium	2O2Y:C	-6.9	

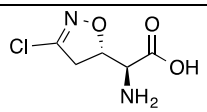
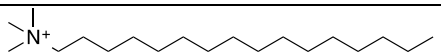
Acivicin	1ZW1:B	-6.5	
Cetrimonium	2OOS:B	-6.3	

Table S1. The list of small molecules from the MicroSource Spectrum and Killer Collection library [1] that were docked into *Pf*ENR. The scores were calculated by AutoDock Vina**.** The PDB ID is given as ID:Chain.

Table S2

	NADH	crotonyl-CoA	Reference
K_m (mM)	0.24 ± 0.04	0.17 ± 0.06	**current study**
k_{cat} (sec^{-1})	0.9 ± 0.1	1.0 ± 0.2	**current study**
k_{cat}/K_m (mM^{-1} sec^{-1})	3.8 ± 1.0	6.0 ± 1.1	**current study**
K_m (mM)	0.03 ± 0.004	0.17 ± 0.015	**Ref 1**
k_{cat} (sec^{-1})	NR	1.62 ± 0.06	**Ref 1**
k_{cat}/K_m (mM^{-1} sec^{-1})	NR	9.8 ± 0.96	**Ref 1**

Table S2. Kinetic parameters calculated for *Pf*ENR. The 100 µL reaction volume contained a final concentration of 0.25 µM ENR, 20 mM Tris/HCl buffer (pH 7.4), with 150 mM NaCl 100 µM crotonyl-CoA, and 100 µM NADH (Sigma). The enzyme was preincubated in 20 mM Tris/HCl buffer (pH 7.4), 150 mM NaCl and crotonyl-CoA, and initiated with NADH to reach its final concentration. The $K_{m,\ crotonyl\text{-}CoA}$ was determined by varying the concentration of crotonyl-CoA while keeping the NADH concentration fixed at 100 µM. Conversely, the $K_{m,\ NADH}$ was determined by varying the concentration of NADH while keeping the crotonyl-CoA concentration fixed at 100 µM.

Figure S1

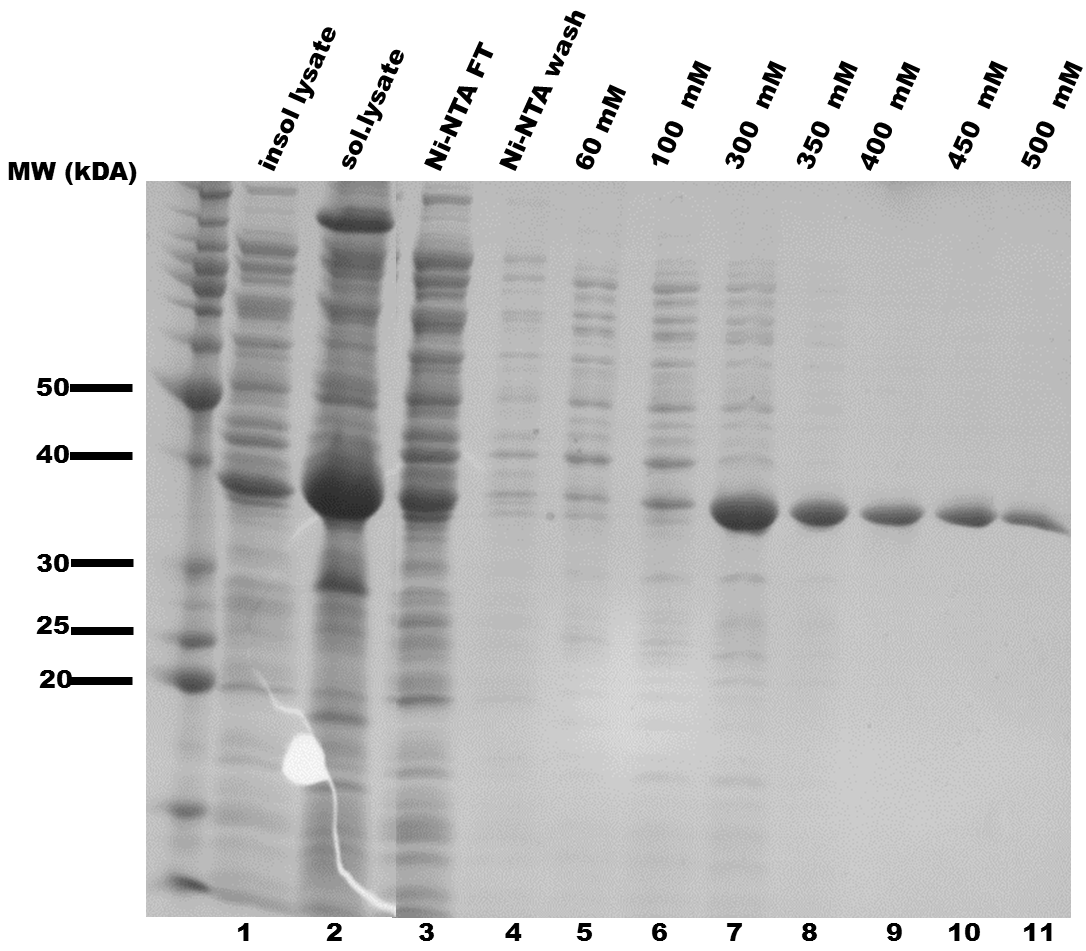


Figure S1. 12% SDS-PAGE showing expression and purification of *P. falciparum* ENR in pET28a plasmid encoding N-terminus 6xHis-tag in *E.coli* BL21 cells. Lane (1) Expression of *Pf*ENR insoluble fraction, (2) soluble fraction, (3) flow-through of Ni-NTA, and (4) buffer wash of Ni-NTA. Lanes (5-11) step gradient of imidazole elution of *Pf*ENR.

Figure S2

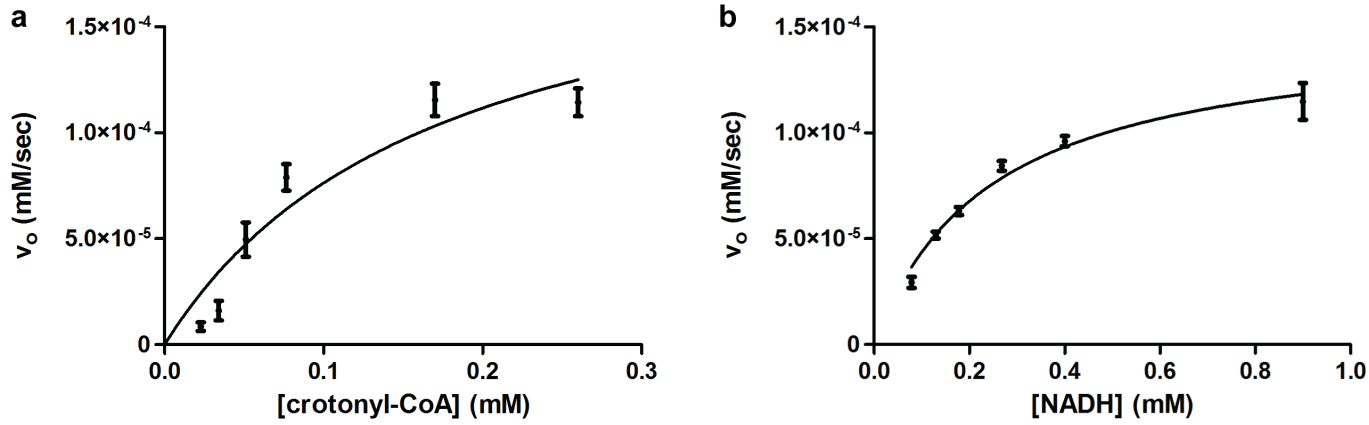


Figure S2. The Michaelis-Menten plots for *P. falciparum* ENR measuring the consumption of NADH at 340 nm (ε_{NADH} = 6.22 mM^{-1} cm^{-1}) for varying concentrations of crotonyl-CoA (a) and NADH (b) at 27 °C. The 100 µL reaction volume contained a final concentration of 0.25 µM ENR, 20 mM Tris/HCl buffer (pH 7.4) with 150 mM NaCl, 100 µM crotonyl-CoA, and 100 µM NADH (Sigma). The enzyme was preincubated in 20 mM Tris/HCl buffer (pH 7.4), 150 mM NaCl, and crotonyl-CoA, and initiated with NADH to reach its final concentration. a) The $K_{m,\ crotonyl\text{-}CoA}$ was determined by varying the concentration of crotonyl-CoA (10-300 µM) while keeping the NADH concentration fixed at 100 µM. b) The $K_{m,\ NADH}$ value was revalidated by titrating NADH (20-500 µM) and keeping the crotonyl-CoA concentration constant at 100 µM. Data was collected in triplicate, and the individual values were within 10% error.

Figure S3

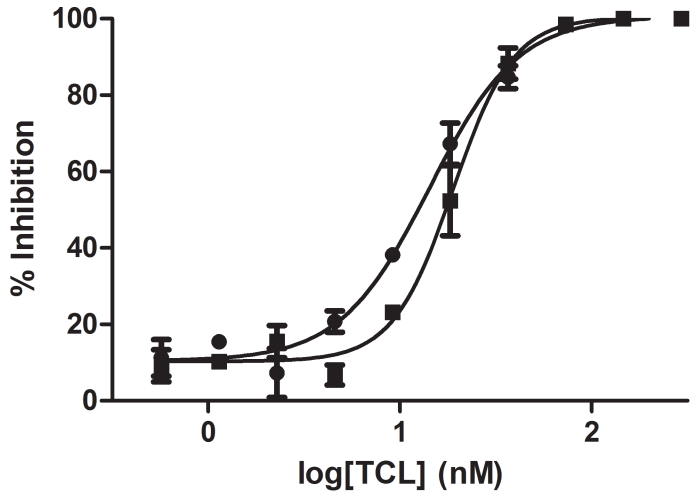


Figure S3. IC_{50} binding curves for triclosan (TCL). The final 100 µL reaction volume contained 50 µM NAD^+, 20 mM Tris/HCl buffer (pH 7.4), 150 mM NaCl, 200 µM crotonyl-CoA, 100 µM NADH, and 0.05 µM ENR. The ENR cocktail was preincubated with a final concentration of 50 µM NAD^+ (Sigma), 20 mM Tris/HCl buffer (pH 7.4), 150 mM NaCl, and varying amounts of triclosan (300 nM to 0.3 nM) (5% v/v DMSO) at 25 °C for 45 minutes. IC_{50} for triclosan (●) without NAD^+ preincubation (■) with 50 µM NAD^+ preincubation. Data was collected in triplicate, and the individual values were within 10% error.

Figure S4

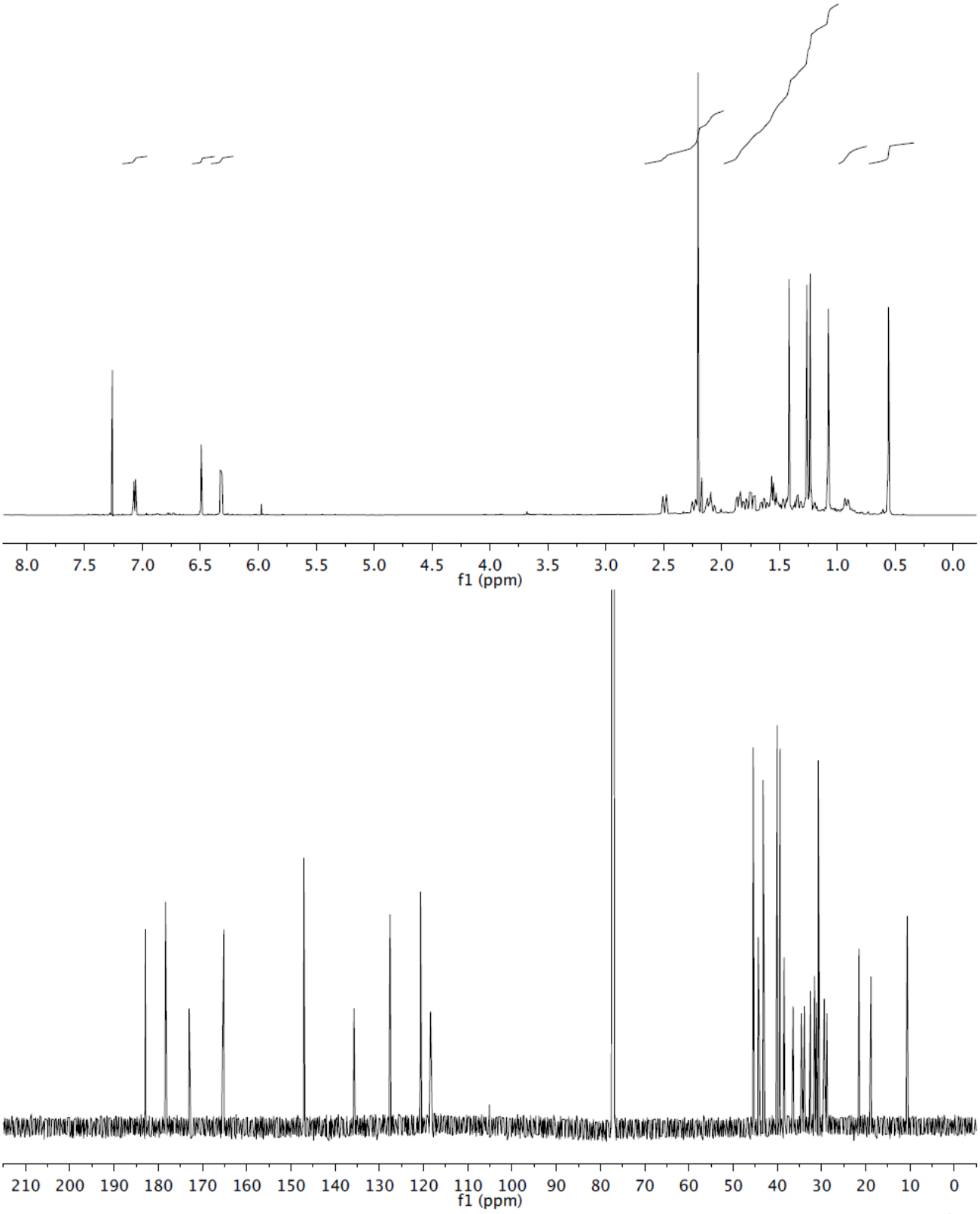


Figure S4. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) of compound **1**.

Figure S5

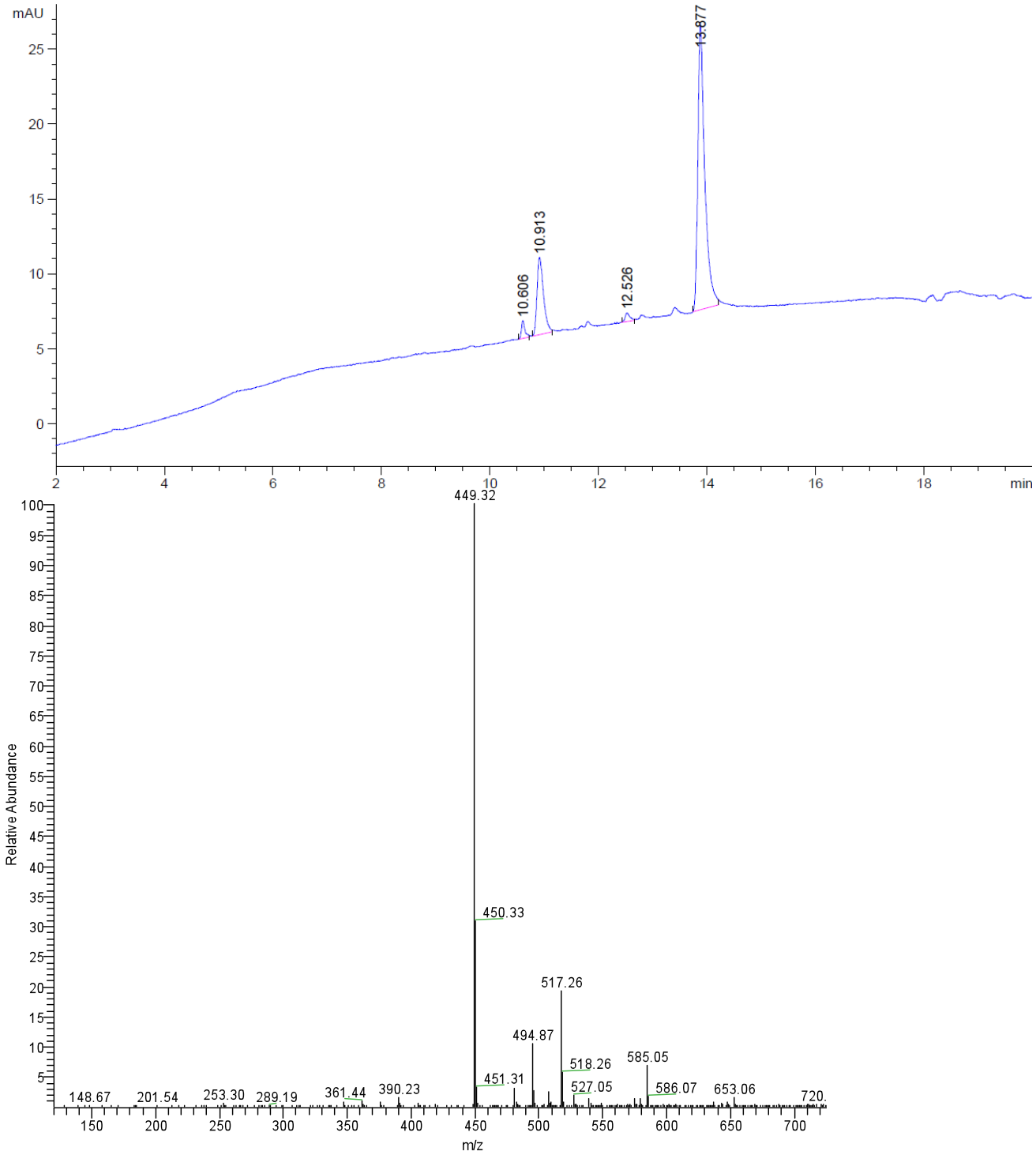


Figure S5. LC-MS Trace of compound **1** (NCI).

Figure S6

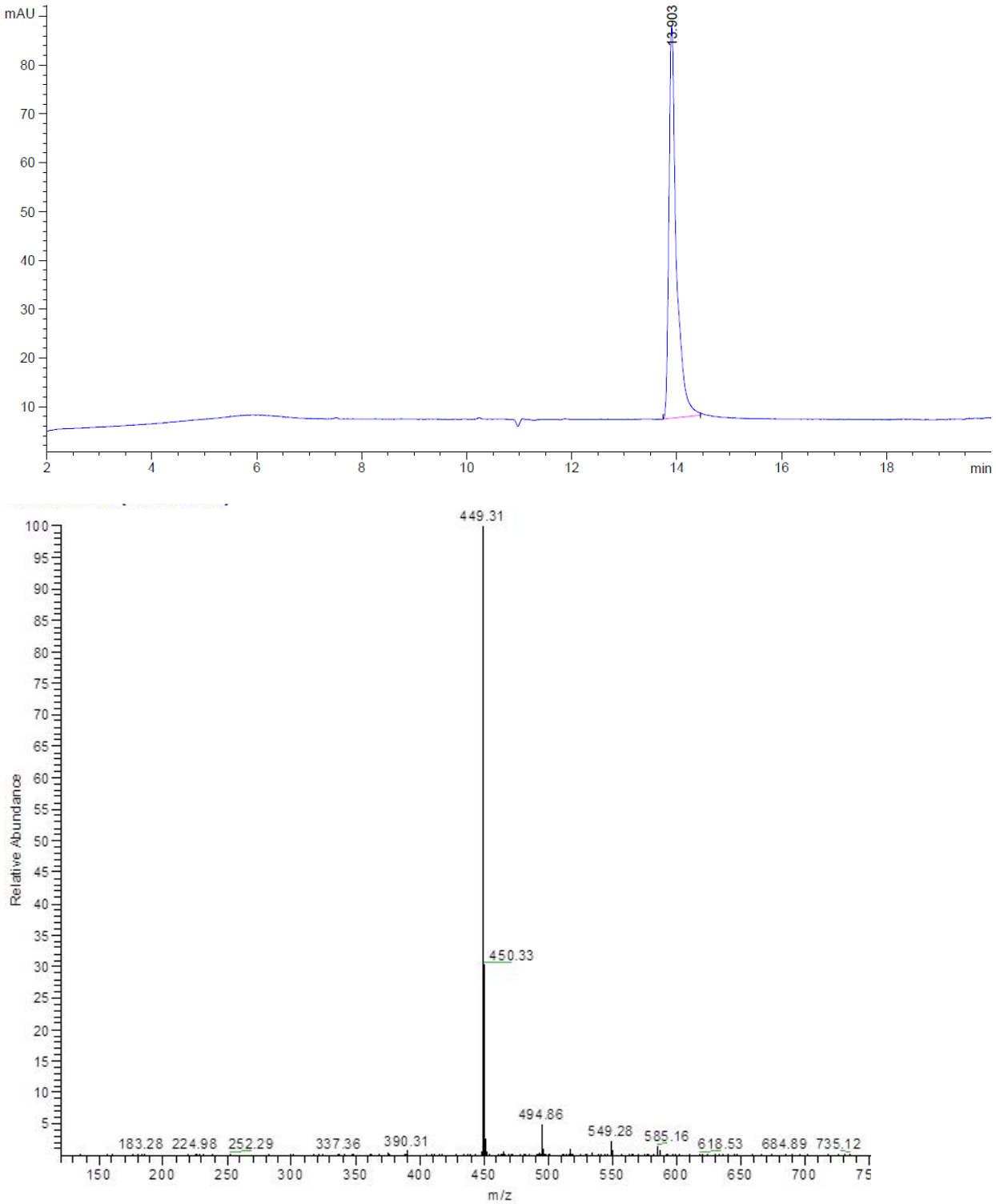


Figure S6. LC-MS Trace of compound **1** (Sigma-Aldrich).

Figure S7.

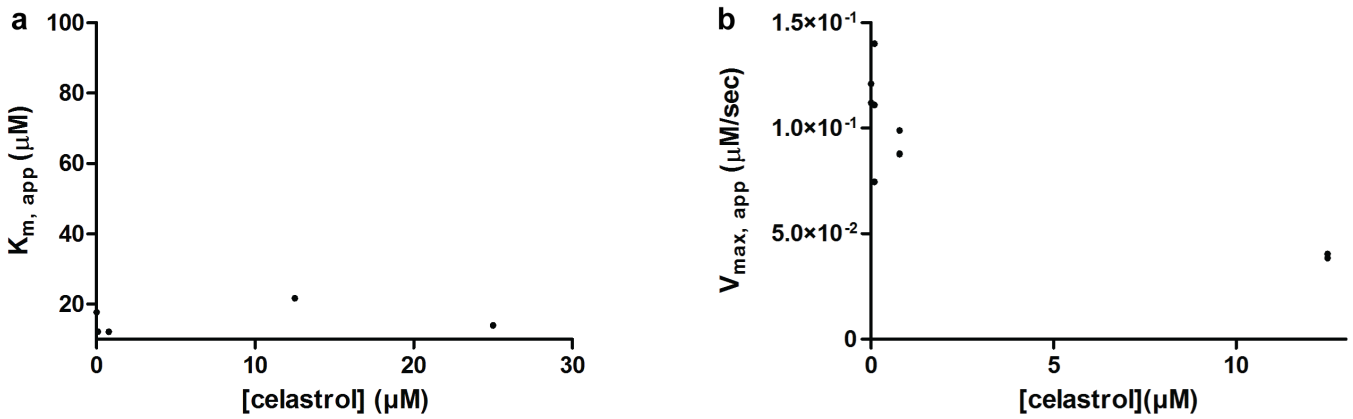


Figure S7. a) $K_{m, app}$ plotted as a function of varying celastrol concentration. $K_{m, app}$ is independent of varying celastrol concentration. b) Scatter plot of $V_{max, app}$ as a function of varying celastrol concentration. The $K_{i, app}$ (5.3 μM) was calculated from the slope of the line (r^2 = 0.89). These experiments were repeated in triplicate.

Figure S8.

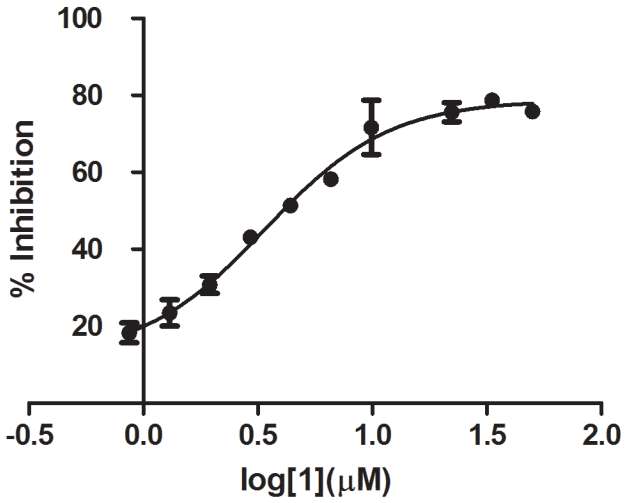


Figure S8. IC_{50} binding curve for celastrol (compound **1**) in the presence of 0.01% Triton-X. The final 100 µL reaction volume contained 50 µM NAD^+, 20 mM Tris/HCl buffer (pH 7.4), 150 mM NaCl, 0.01% Triton-X, 200 µM crotonyl-CoA, 100 µM NADH, and 0.05 µM ENR. ENR was preincubated at 25 °C for 45 minutes with a final concentration of 50 µM NAD^+, 20 mM Tris/HCl buffer (pH 7.4), 150 mM NaCl, and varying concentrations (0.9-50 µM) of **1**. Data was collected in duplicate, and the individual values were within 10% error.

Figure S9.

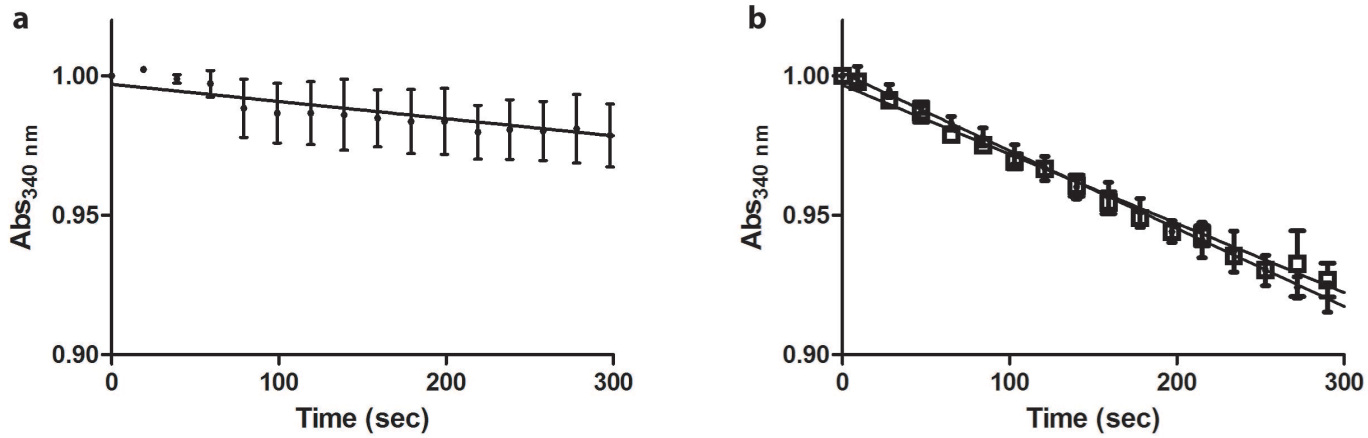


Figure S9. A comparison between the activity of *Pf*ENR a) with and without b) rapid dilution. a) The final 100 µL reaction volume contained 50 µM NAD^+, 20 mM Tris/HCl buffer (pH 7.4), 150 mM NaCl, 0.01% Triton-X, 200 µM crotonyl-CoA, 100 µM NADH, 15 µM of compound **1**, and 0.05 µM ENR. Data was collected in duplicate with r^2 values greater than 0.97. b) 3 µM *Pf*ENR was incubated with 15 µM of compound **1** and 5 mM NAD^+ for 45 minutes at 25 °C. The protein was diluted 100-fold with 200 µM crotonyl-CoA and 100 µM NADH to initiate the reaction. The two lines represent the activity of *Pf*ENR with celastrol (solid circle) and DMSO (open square). This experiment was conducted in triplicate with r^2 values greater than 0.97.

Figure S10.

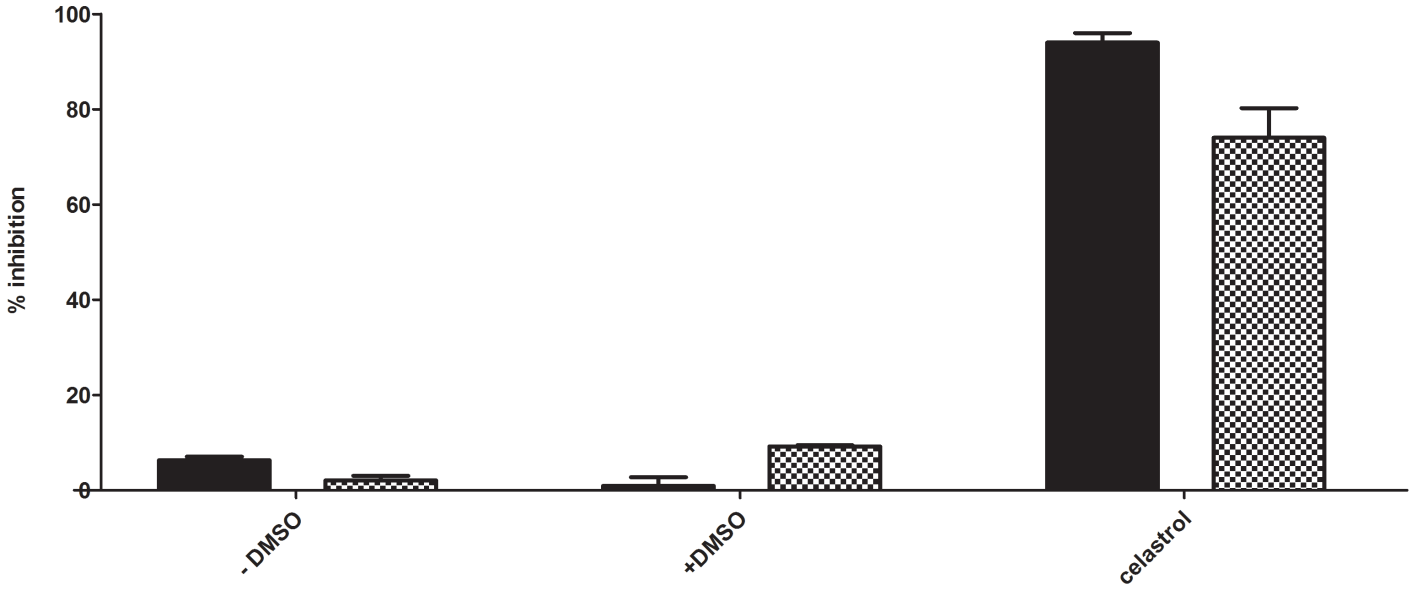


Figure S10. Comparison of *Pf*ENR inhibition without iodoacetamide treatment (solid black) and with iodoacetamide treatment (checkered box). The final 100 µL reaction volume contained 50 µM NAD^+, 20 mM Tris/HCl buffer (pH 7.4), 150 mM NaCl, 0.01% Triton-X, 200 µM crotonyl-CoA, 100 µM NADH, 50 µM of compound **1**, and 0.05 µM ENR. These experiments were conducted in triplicate.

Figure S11

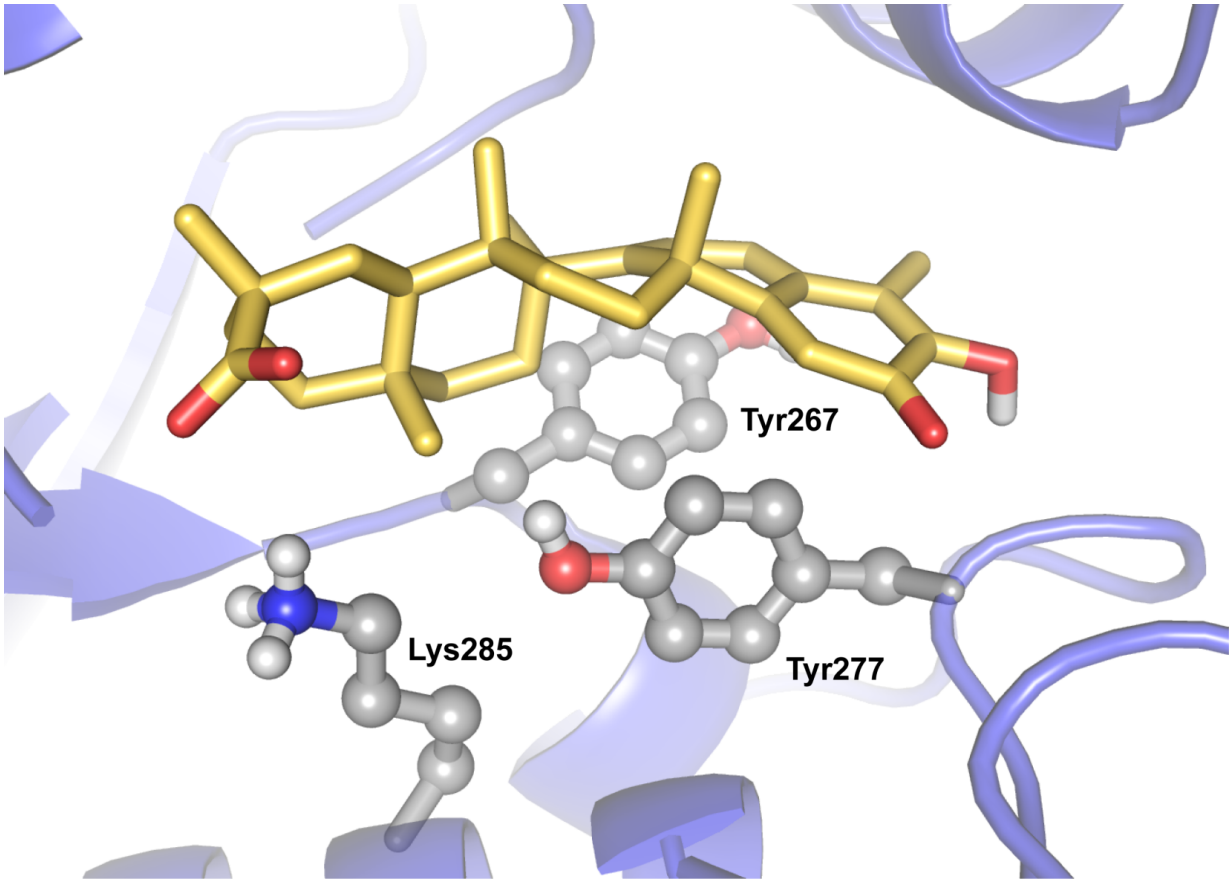


Figure S11. The best-predicted celastrol docking pose, obtained when the molecule was docked into the 2OL4 structure. Part of the protein has been removed to facilitate visualization.