Supporting Information for

Mechanism and Inhibition of saFabI, the Enoyl Reductase from *Staphylococcus aureus*[†]

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Table S1 Nucleotide Primers

Name	Sequence [a]
saFabI forward	5'GAGA*CATATG*TTAAATCTTGAAAACAAAACTTATGTCATCATGGG 3'
saFabI reverse	5' CTC*GGATCC*AATAACGTGAACAAAGCTGTTGAATG 3'
saACP forward	5' GGAATTC*CATATG*GTGGAAAATTTCGATAAAG 3'
saACP reverse	5' CCG*CTCGAG*TTTTTCAAGACTGTTAATAAA 3'
A95V forward	5' GTGTATATCATTCAATCGTATTTGCTAATATGGAAG 3'
A95V reverse	5' CTTCCATATTAGCAAATACGATTGAATGATATACAC 3'
I193S forward	5' CAGCTAGTCCAAGCCGTACATTAAG 3'
I193S reverse	5' CTTAATGTACGGCTTGGACTAGCTG 3'
F204S forward	5' CAAAAGGTGTGGGTGGTTCCAATACAATTCTTAAAG 3'
F204S reverse	5' CTTTAAGAATTGTATTGGAACCACCCACACCTTTTG 3'
R40Q forward	5' GTATTTACTTACCAGAAAGAACGTAGCCG 3'
R40Q reverse	5' CGGCTACGTTCTTTCTGGTAAGTAAATAC 3'
K41N forward	5' GTATTTACTTACCGTAACGAACGTAGCCGTAAAG 3'
K41N reverse	5' CTTTACGGCTACGTTCGTTACGGTAAGTAAATAC 3'
R40Q/K41N forward	5' GTATTTACTTACCAGAACGAACGTAGCCGTAAAG 3'
R40Q/K41N reverse	5' CTTTACGGCTACGTTCGTTCTGGTAAGTAAATAC 3'

[a] Restriction sites are italicized, and mutated sites are shown in underline.

Table S2: MICs of diphenyl ethers against different strains of *S. aureus*

Strains	MIC μg/mL (μM)		
	Triclosan	EPP	CPP
ATCC 29213	0.03 (0.10)	0.03 (0.14)	0.03 (0.14)
N315	0.03 (0.10)	0.03 (0.14)	0.06 (0.27)
Mu50	0.06 (0.20)	0.06 (0.27)	0.12 (0.54)

Table S3: MICs of diphenyl ethers against *S. aureus* and *E. faecalis*

Compound	MIC (μg/mL)	
	S. aureus ATCC 25923	*E. faecalis* ATCC 19433
Triclosan	0.03	>32
EPP	0.03	>32
CPP	0.06	>32

Figure Legends for Supplemental Information

Figure S1: Fluorescence titration of saFabI with apo-saACP. The excitation wavelength was 290 nm, and the emission wavelength was 336 nm.

Figure S2: Double Reciprocal Plots for the Reaction of DDsaACP and NADPH with Wild Type saFabI (A) Double reciprocal plot obtained by varying DDsaACP in the presence of 250 μM NADPH (○), 100 μM NADPH (●) and 50 μM NADPH (□). (B) Double reciprocal plot obtained by varying NADPH in the presence of 15 μM DDsaACP (○), 8.2 μM DDsaACP (●), and 4.4 μM DDsaACP (□).

Figure S3: Double Reciprocal Plots Showing the Effect of $NADP^+$ on the Reaction of DDsaACP and NADPH with Wild Type saFabI (A) Reactions were carried out in the absence of $NADP^+$ (○), or in the presence of 1.4 mM $NADP^+$ (●) and 2.7 mM $NADP^+$ (□). (B) Reactions were carried out in the absence of $NADP^+$ (○), or in the presence of 0.7 mM $NADP^+$ (●) and 1.4 mM $NADP^+$ (□).

Figure S4: Fluorescence Titration of saFabI with DDsaACP Excitation wavelength is 290 nm, and emission wavelength is 336 nm.

Figure S5. Fluorescence Titration of saFabI with the Diphenyl Ether Inhibitors Fluorescence titration of saFabI with triclosan (A), EPP (B) and CPP (C), respectively. The excitation wavelength was 290 nm, and the emission wavelength was 336nm.

Figure S6: Inhibition of A95V saFabI by the Diphenyl Ethers (A) Triclosan: 0 (○), 11.0 (●), 38.6 (□) and 65.0 μM (■) triclosan. (B) EPP: 0 (○),0.9 (●), 1.8 (□) and 5.0 μM (■) EPP. (C) CPP: 0 (○), 3.1 (●), 6.2 (□) and 12.4 μM (■) CPP.

Figure S7: Inhibition of I193S saFabI by the Diphenyl Ethers (A) Triclosan: 0 (○),0.22 (●), 0.44 (□) and 0.88 μM (■) triclosan. (B) EPP: 0 (○), 0.21 (●) and 0.35 μM (□) EPP. (C) CPP: 0 (○), 0.31 (●) and 0.62 μM (□) CPP.

Figure S8: Inhibition of F204S saFabI by the Diphenyl Ethers (A) Triclosan: 0 (○), 0.28 (●), 0.55 (□) and 1.10 μM (■) triclosan. (B) EPP: 0 (○), 0.24 (●), 0.47 (□) and 1.10 μM (■) EPP. (C) CPP: 0 (○), 0.64 (●), 1.28 (□) and 2.48 μM (■) CPP.

Figure S1: Fluorescence titration of saFabI with apo-ACP.

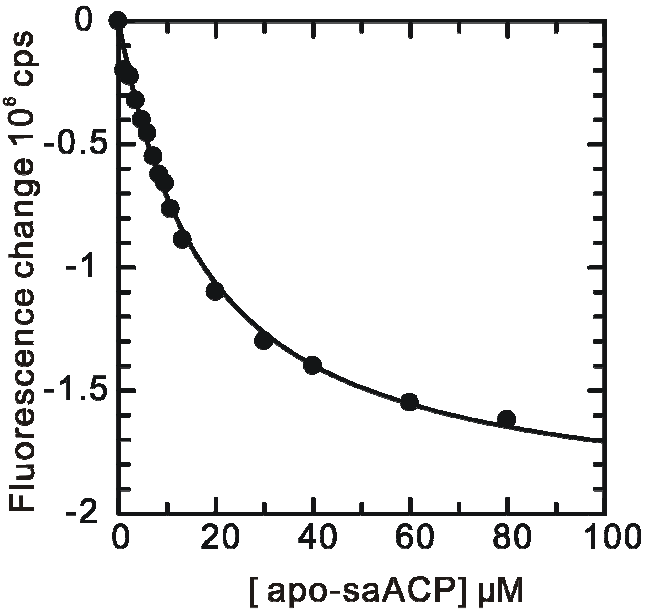

Figure S2: Double Reciprocal Plots for the Reaction of DDsaACP and NADPH with Wild Type saFabI

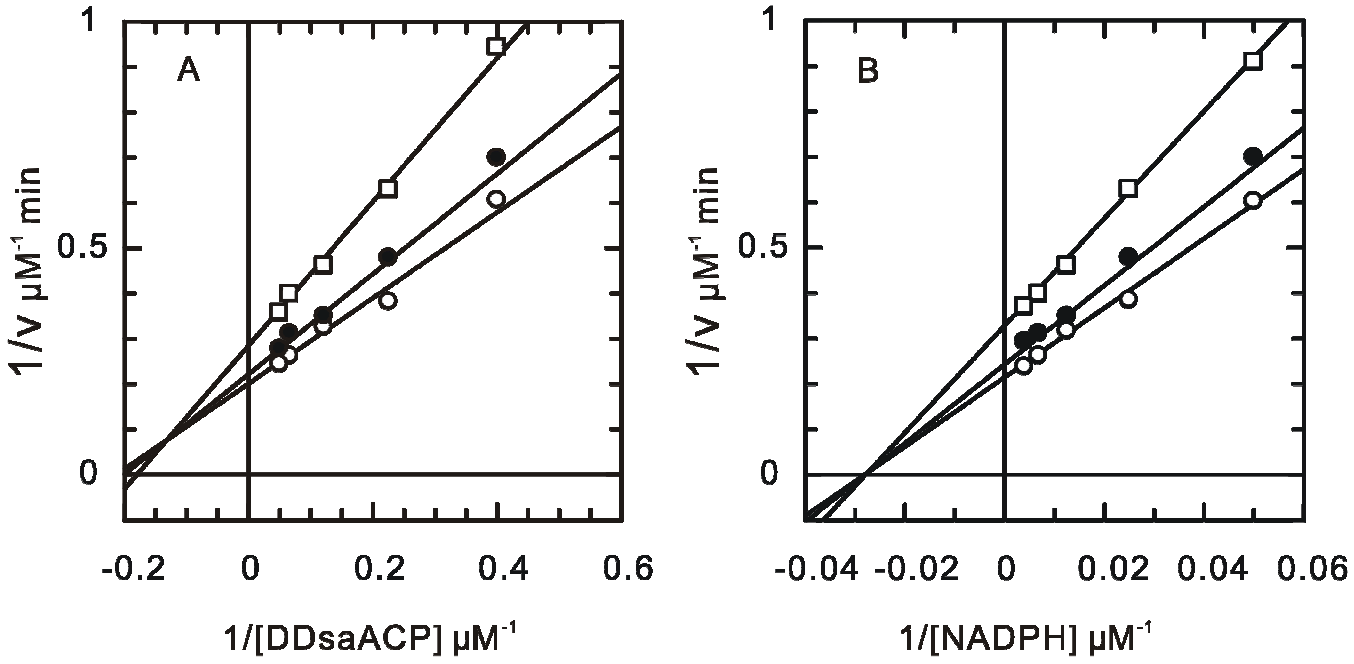

Figure S3: Double Reciprocal Plots Showing the Effect of $NADP^+$ on the Reaction of DDsaACP and NADPH with Wild Type saFabI

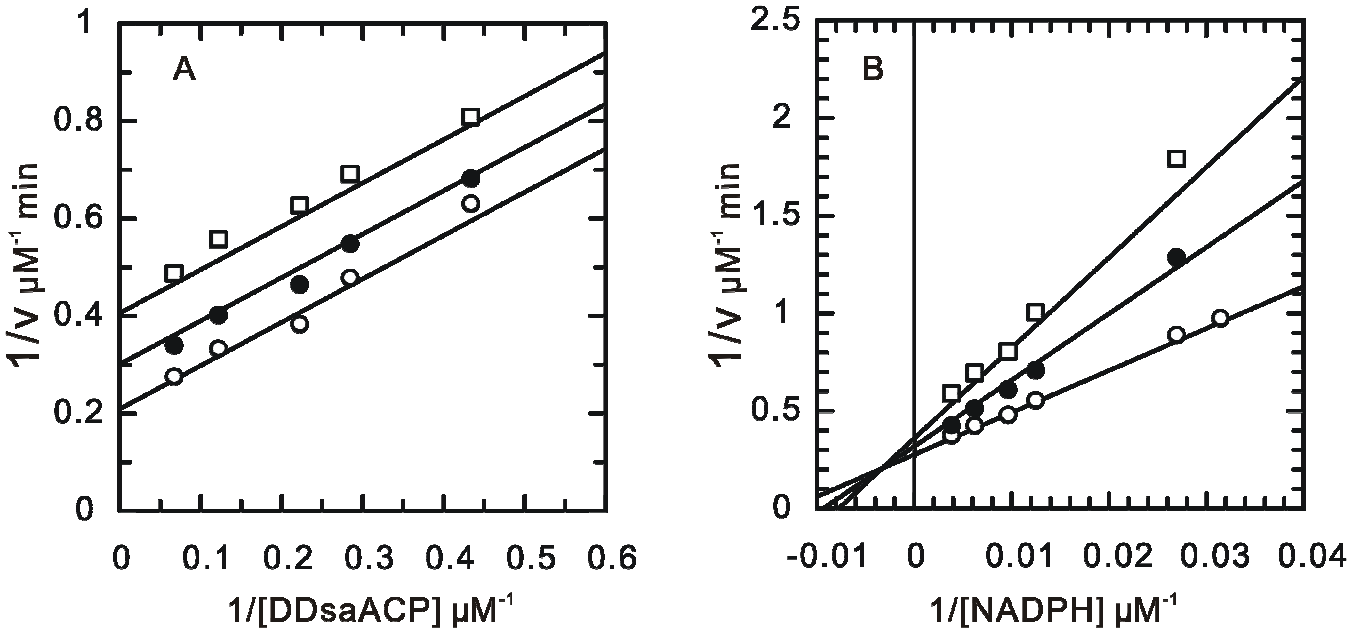

Figure S4: Fluorescence Titration of saFabI with DDsaACP.

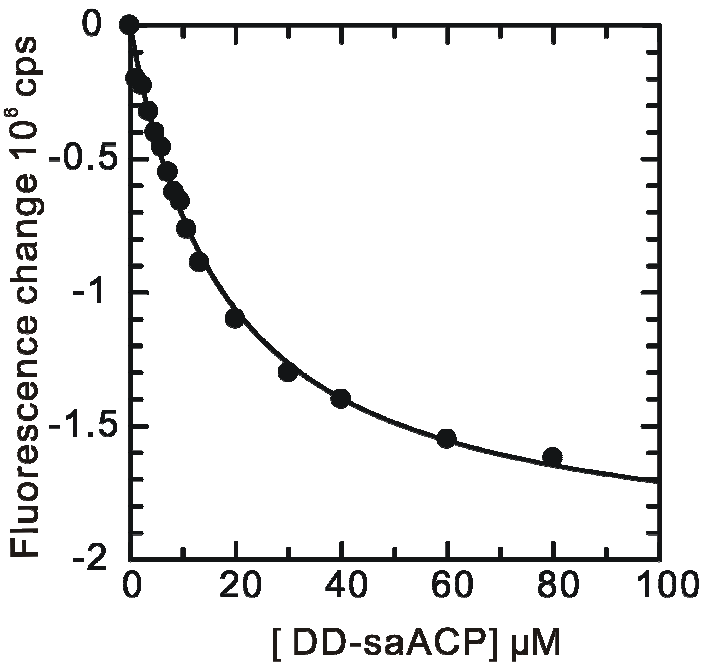

Figure S5. Fluorescence Titration of saFabI with the Diphenyl Ether Inhibitors.

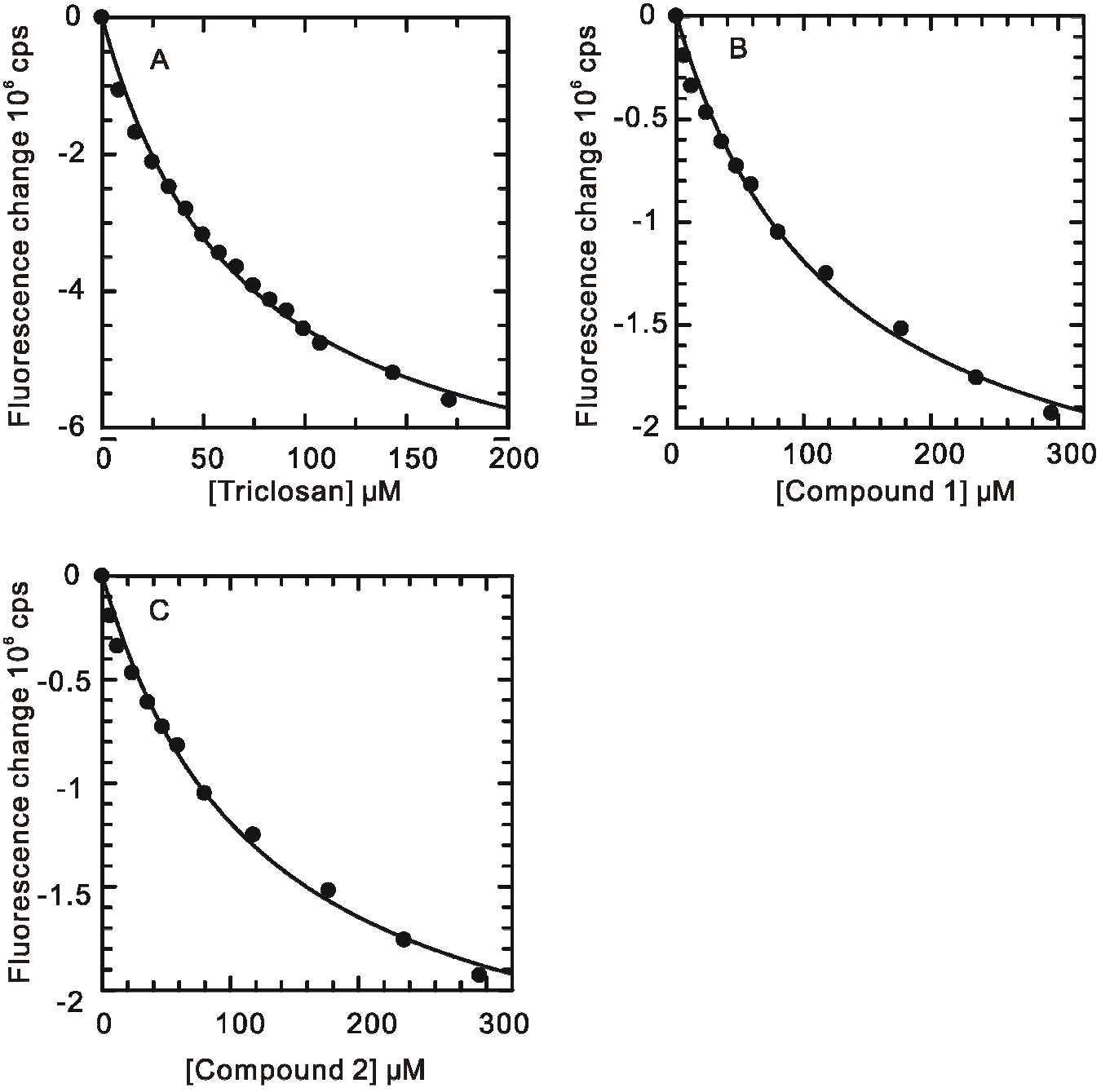

Figure S6: Inhibition of A95V saFabI by the Diphenyl Ethers.

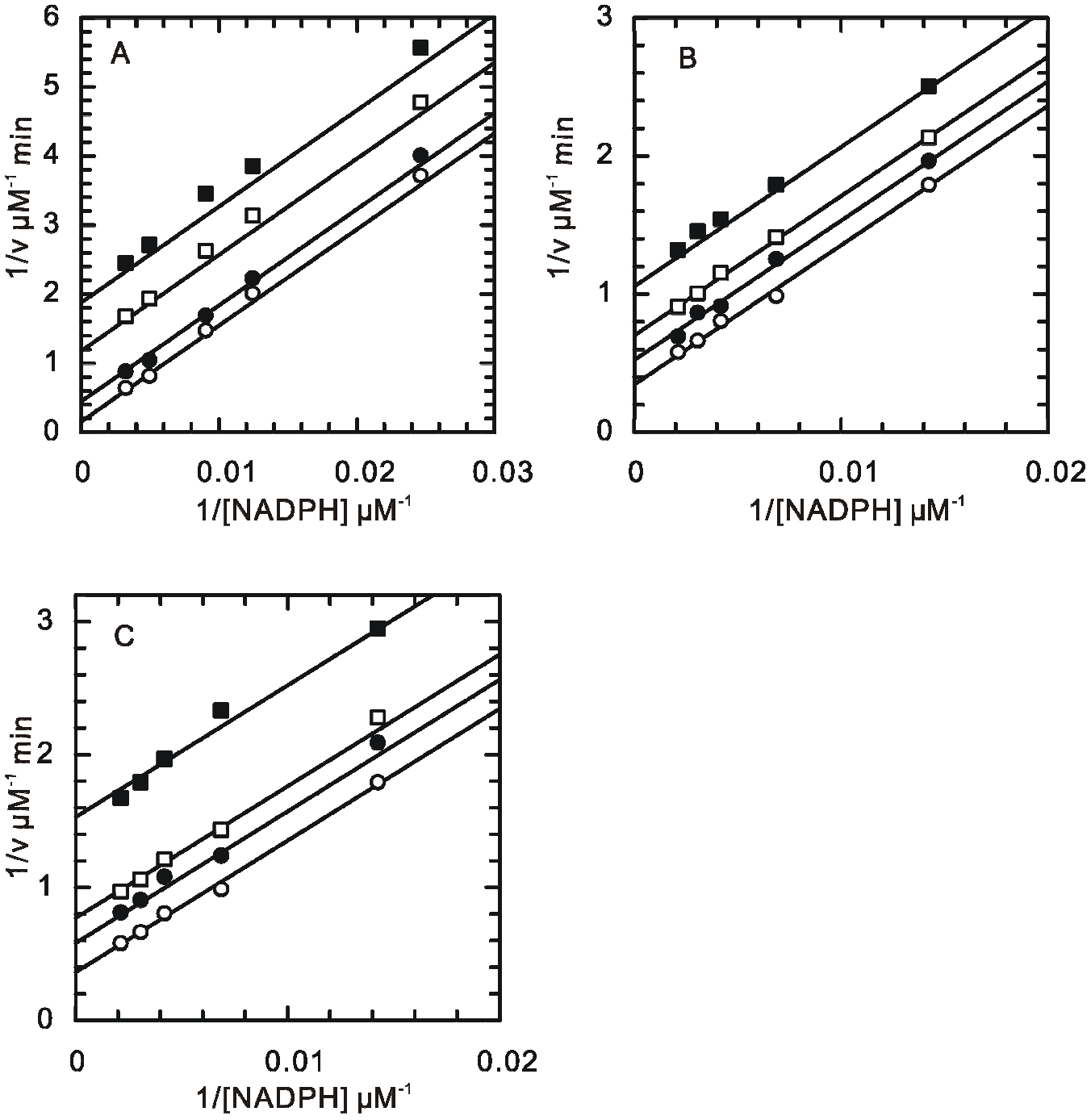

Figure S7: Inhibition of I193S saFabI by the Diphenyl Ethers.

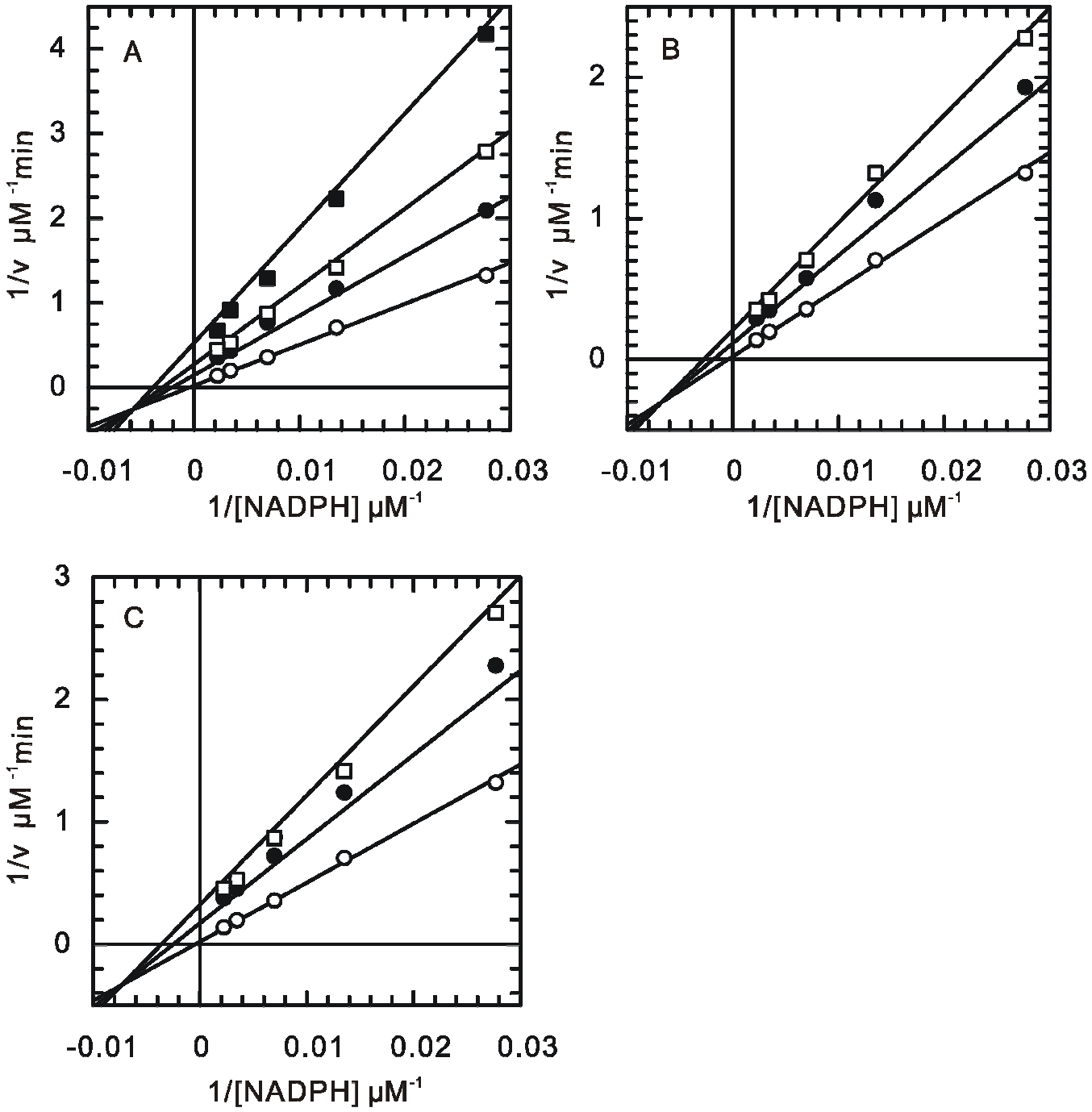

Figure S8: Inhibition of F204S saFabI by the Diphenyl Ethers.