Supporting Information for:

Identification and Analysis of Small Molecule Inhibitors of FosB from *Staphylococcus aureus*

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Table S1. Data collection and structure refinement statistics of *B. cereus* FosB in complex with SO4, PPA, 2-PPP, HPP, H2Y, H2M.

Inhibitor bound	-	PPA	2-PPP	HPP	H2Y	H2M
PDB entry	8G71	8E7R	8E7Q	8G7F	8G7H	8G7G
Data collection						
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions						
a, *b*, *c* (Å)	63.5, 68.4, 70.1	64.7, 67.8, 69.7	64.3, 68.0, 69.8	64.2, 68.3, 69.9	64.2, 68.2, 69.8	64.2, 68.5, 69.5
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	50.00 – 1.83 (1.86 – 1.83)	50.00 – 1.98 (2.01 – 1.98)	50.00 – 1.9 (1.93 – 1.9)	50.00 – 2.04 (2.08 – 2.04)	50.00 – 1.81 (1.84 – 1.81)	50.00 – 2.23 (2.27 – 2.23)
R_{sym}	0.140 (0.782)	0.16 (0.583)	0.141 (0.570)	0.164 (0.746)	0.138 (0.785)	0.144 (0.552)
No. of unique reflections	27944 (1388)	22012 (1054)	24190 (1182)	20157 (1012)	28683 (1409)	15452 (738)
$I/\sigma I$	16.8 (2.2)	9.4 (1.9)	10.2 (2.0)	9.1 (1.8)	17.0 (2.1)	10.38 (2.1)
$CC_{1/2}$	0.99 (0.87)	0.98 (0.75)	0.98 (0.78)	0.99 (0.79)	0.99 (0.84)	0.99 (0.85)
Completeness (%)	99.7 (99.9)	99.8 (99.8)	97.6 (98.3)	99.9 (100)	99 (100)	99.7 (98.5)
Average redundancy	10.6 (10.4)	5.2 (5.0)	5.0 (5.1)	7.3 (7.4)	10.7 (9.5)	7.8 (6.6)
Structure refinement						
Resolution (Å)	48.94 – 1.82	46.81 – 1.97	38.83 – 1.90	47.27 – 2.00	48.80 – 1.81	47.20 – 2.23
R_{work} / R_{free} (%)	15.9 / 18.8	16.9 / 22.0	16.9 / 21.9	16.1 / 21.5	16.0 / 19.0	17.9 / 24.5
r.m.s. deviations						
Bond lengths (Å)	0.013	0.007	0.008	0.009	0.010	0.008
Bond angles (°)	1.536	1.444	1.489	1.483	1.686	1.456
Ramachandran plot statistics:[a]						
% of residues in favored region	98.2	97.8	98.5	97.4	99.3	97.4
% of residues in allowed region	1.8	2.2	1.5	2.6	0.7	2.6
% outliers	0	0	0	0	0	0
Additional ligands in the structure (PDB chemical ID)	Sulfate ion (SO4) Zinc ion (Zn) Magnesium ion (Mg) Formic acid (FMT)	Zinc ion (Zn) Magnesium ion (Mg) Formic acid (FMT)	Zinc ion (Zn) Magnesium ion (Mg) Formic acid (FMT)	Zinc ion (Zn) Magnesium ion (Mg) Formic acid (FMT) Glycerol (GOL)	Zinc ion (Zn) Magnesium ion (Mg) Formic acid (FMT) Glycerol (GOL)	Zinc ion (Zn) Magnesium ion (Mg) Formic acid (FMT)

Values in the parentheses were calculated from data for the highest resolution shell. [a]The stereochemistry of the refined structures was assessed using MolProbity[1]

Table S2. Molecular interactions of ligands in the $FosB^{Bc}$ active site.

Compound	Molecular Interactions	Distance (Å)
Fosfomycin (PDB ID: 4JH3)	Tyr64:OH—Lig:O3	2.6
	Arg94:NE—Lig:O3	2.8
	Arg94:NH2—Lig:O1	2.9
	Tyr105:OH—Lig:O2	2.6
	Arg124:NH1—Lig:O1	2.9
	Zn—Lig:O2	2.0
	Zn—Lig:O	2.5

PPF (PDB ID: 8DTD)	Asn50:NH—Lig:O4	3.4
	Tyr64:OH—Lig:O3	2.4
	Arg94:NE—Lig:O3	2.8
	Arg94:NH2—Lig:O1	2.8
	Tyr105:OH—Lig:O2	2.5
	Arg124:NH1—Lig:O1	3.0
	Zn—Lig:O2	2.1
	Zn—Lig:O5	2.2
1 (PDB ID: 8G7F)	Asn50:NH—Lig:O1	2.8
	Tyr64:OH—Lig:O3	2.7
	Arg94:NE—Lig:O3	2.9
	Arg94:NH2—Lig:O2	3.0
	Tyr105:OH—Lig:O4	2.7
	Arg124:NH1—Lig:O2	2.8
	Zn—Lig:O4	1.9
2 (PDB ID: 8G7H)	Tyr39:OH—Lig:O1	2.7
	Tyr64:OH—Lig:O2	2.6
	Arg94:NE—Lig:O2	2.9
	Arg94:NH2—Lig:O4	2.8
	Tyr105:OH—Lig:O3	2.8
	Arg124:NH1—Lig:O4	2.9
	Zn—Lig:O3	1.9
3 (PDB ID: 8G7G)	Tyr64:OH—Lig:O4	2.6
	Arg94:NE—Lig:O4	3.0
	Arg94:NH2—Lig:O3	2.8
	Tyr105:OH—Lig:O2	2.5
	Arg124:NH1—Lig:O3	3.0
	Zn—Lig:O2	2.0
	Zn—Lig:O1	2.4

	Asn50:NH—Lig:O2	2.6
	Tyr64:OH—Lig:O3	2.6
	Arg94:NE—Lig:O3	3.0
4	Arg94:NH2—Lig:O5	2.8
(PDB ID: 8E7R)	Tyr105:OH—Lig:O4	2.8
	Arg124:NH1—Lig:O5	2.9
	Zn—Lig:O4	1.9
	Zn—Lig:O1	3.4
	Asn50:NH—Lig:O2	3.1
	Tyr64:OH—Lig:O4	2.6
	Arg94:NE—Lig:O4	3.1
5	Arg94:NH2—Lig:O3	2.9
(PDB ID: 8E7Q)	Tyr105:OH—Lig:O5	2.6
	Arg124:NH1—Lig:O3	2.8
	Zn—Lig:O5	2.0
	Zn—Lig:O1	4.4

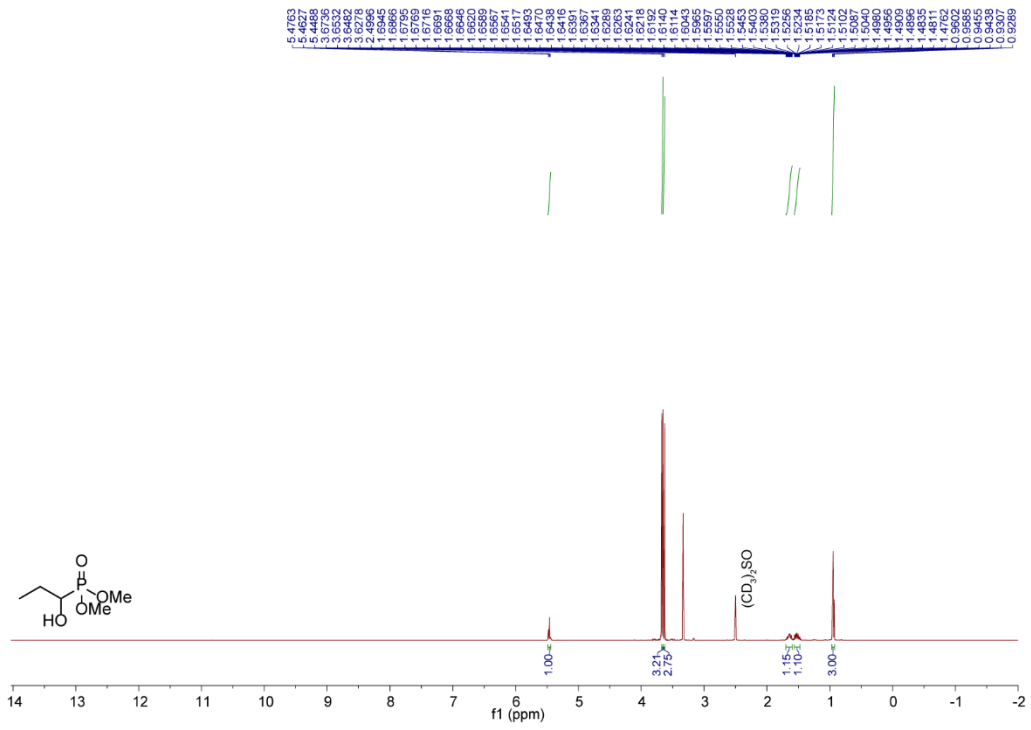


Figure S1. ^{1}H NMR spectrum for compound **9** in $(CD_3)_2SO$ (500 MHz).

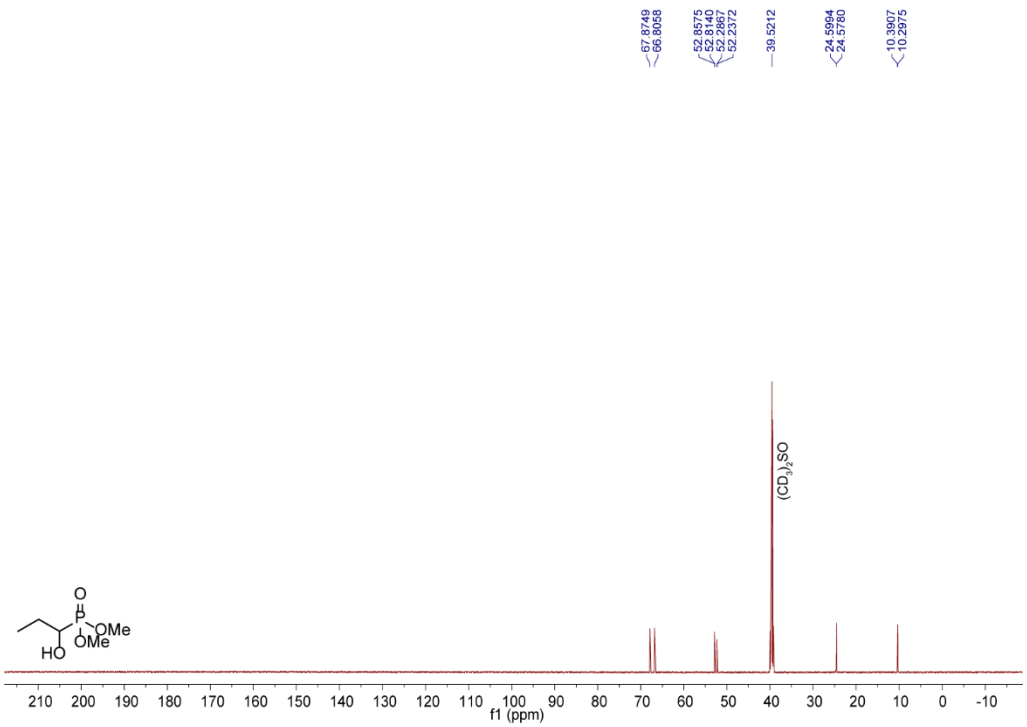


Figure S2. ^{13}C NMR spectrum for compound **9** in $(CD_3)_2SO$ (150 MHz).

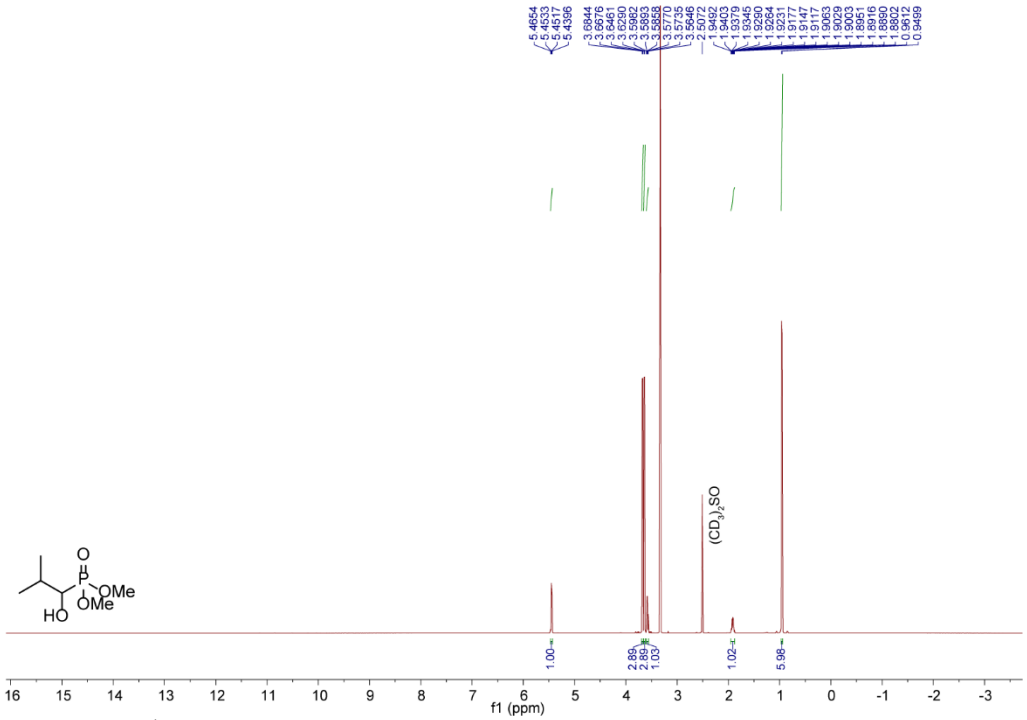


Figure S3. ^{1}H NMR spectrum for compound **10** in $(CD_3)_2SO$ (600 MHz).

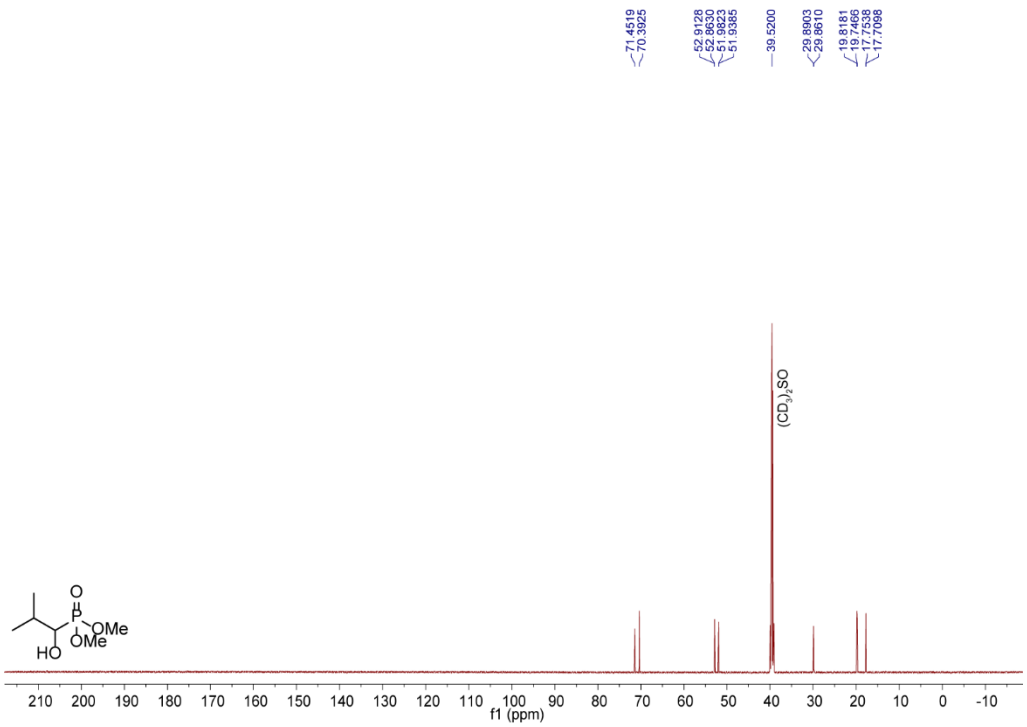


Figure S4. ^{13}C NMR spectrum for compound **10** in $(CD_3)_2SO$ (150 MHz).

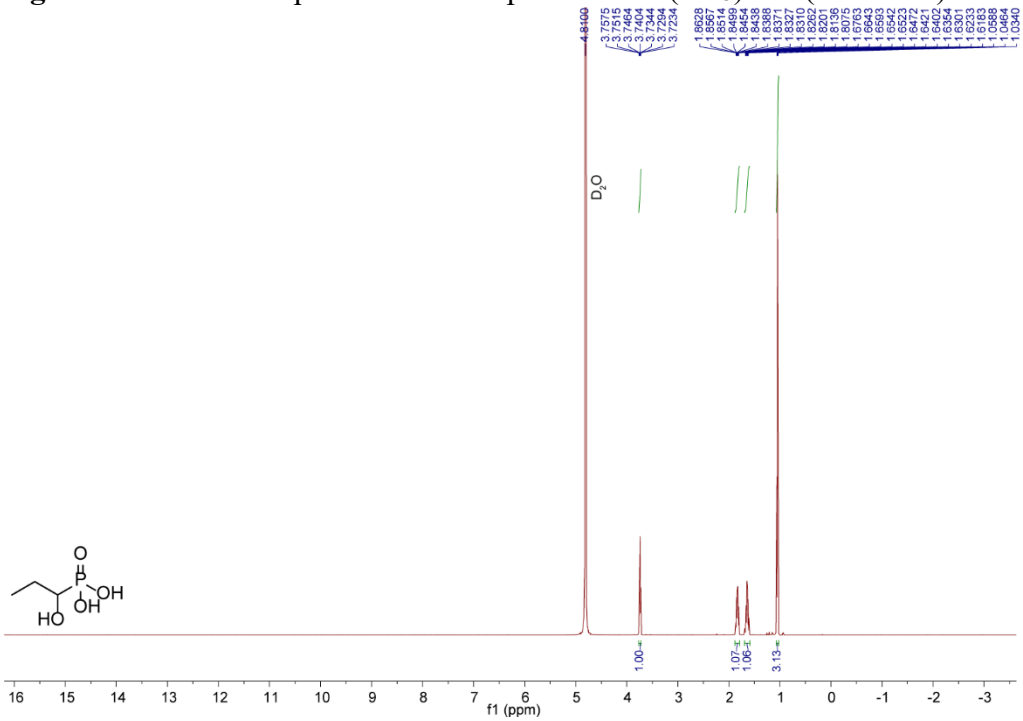


Figure S5. ^{1}H NMR spectrum for compound **1** (1H2M) in D_2O (600 MHz).

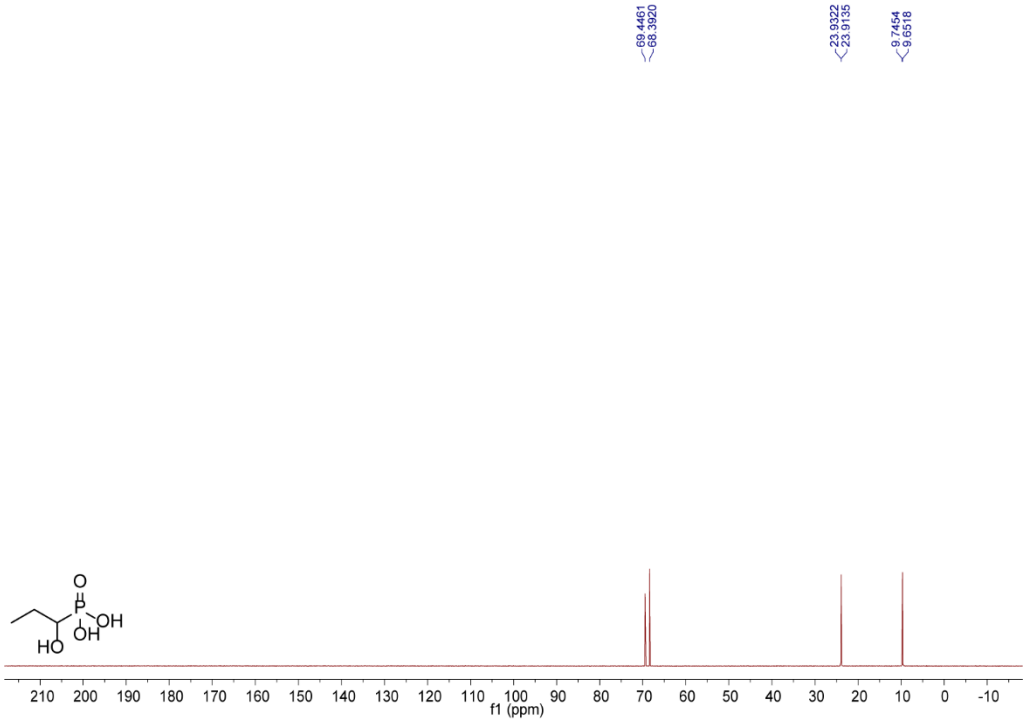


Figure S6. ^{13}C NMR spectrum for compound **1** (1H2M) in D_2O (150 MHz).

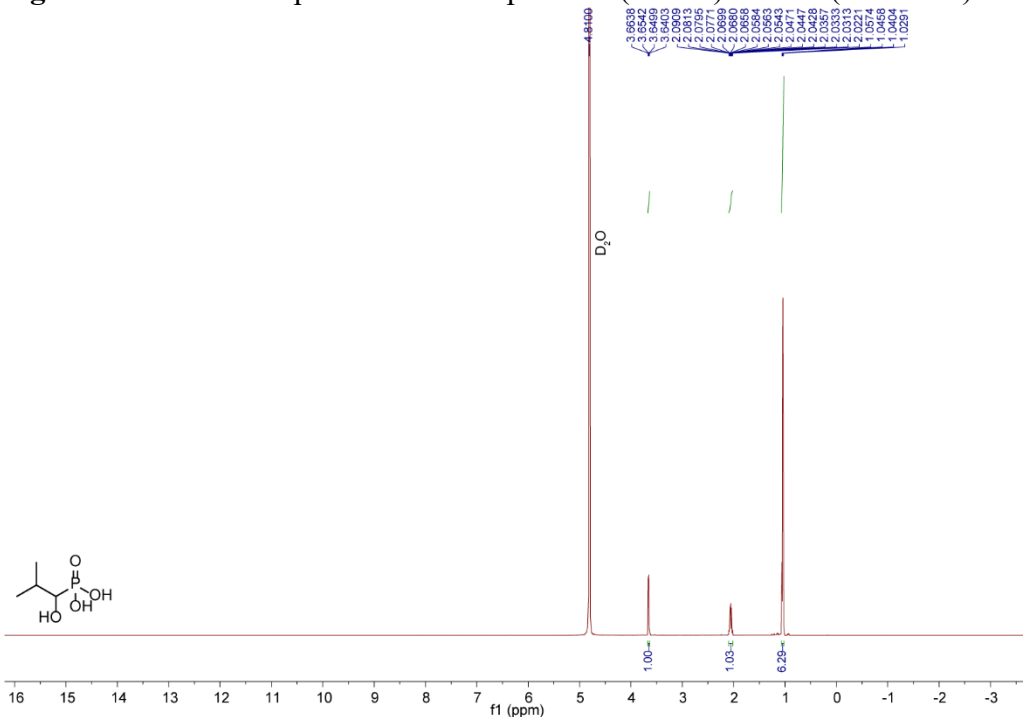


Figure S7. ^{1}H NMR spectrum for compound **3** (1HPP) in D_2O (600 MHz).

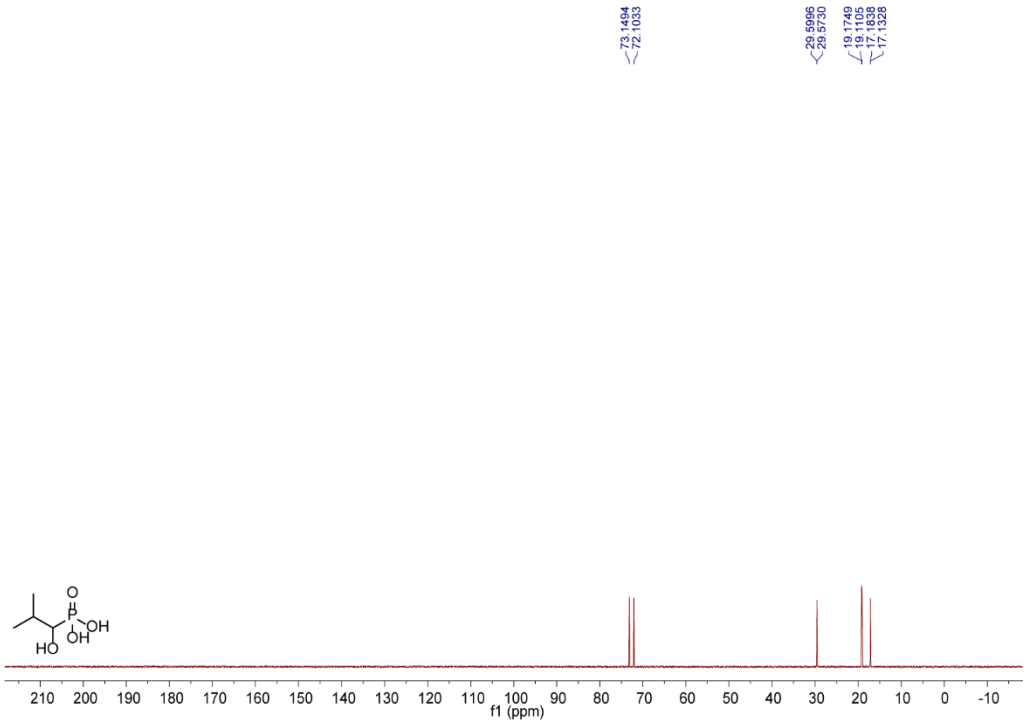
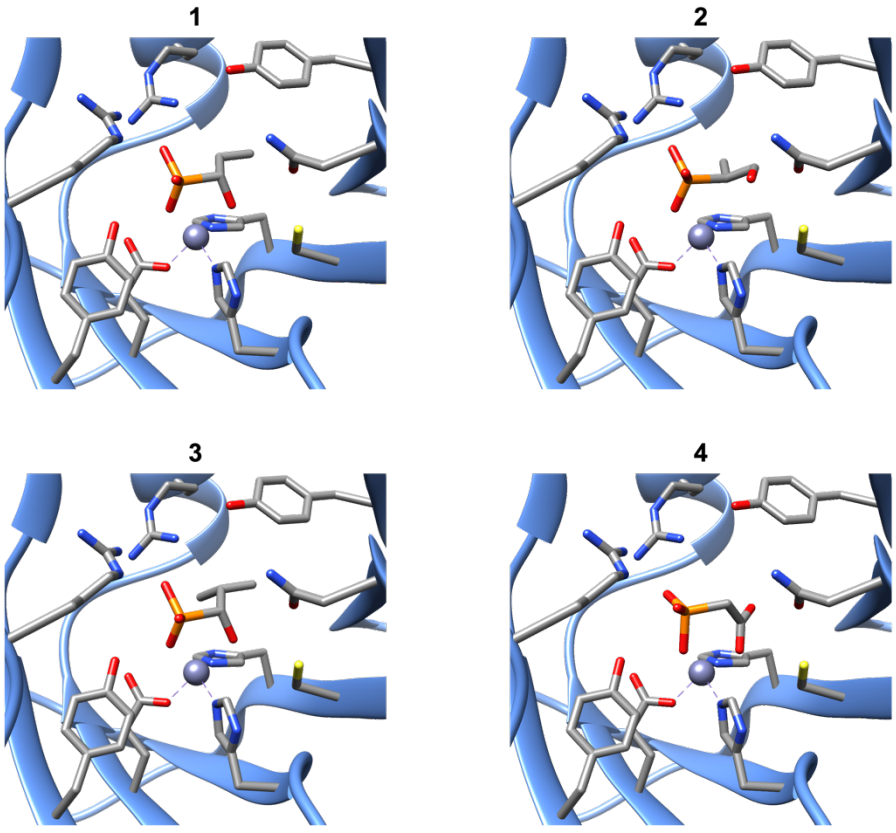


Figure S8. ^{13}C NMR spectrum for compound **3** (1HPP) in D_2O (150 MHz).



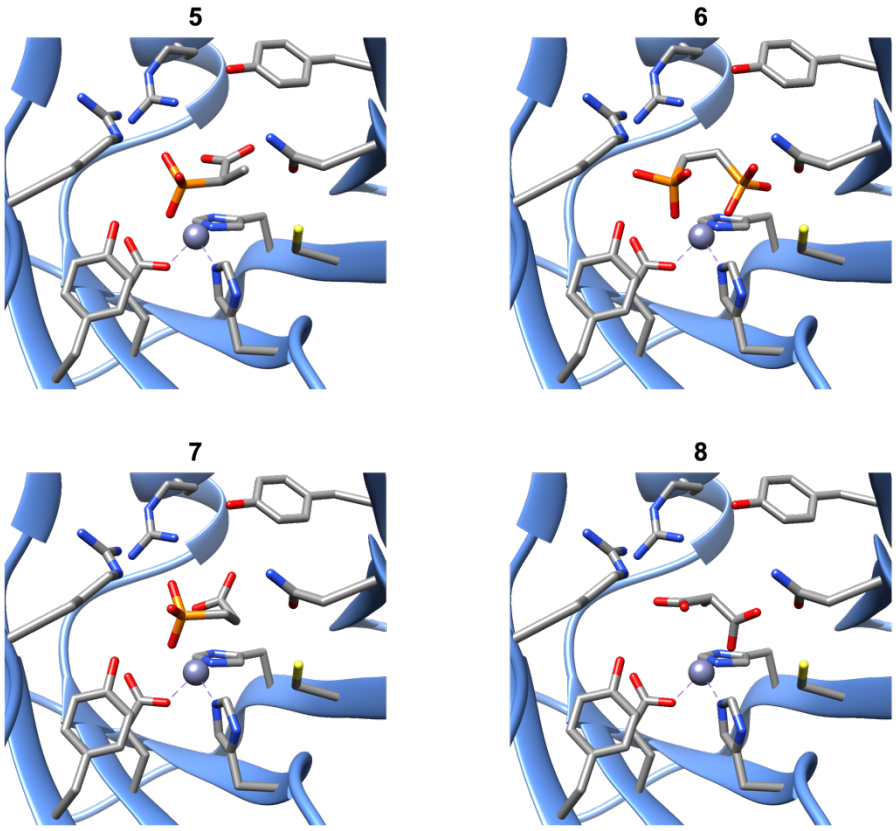
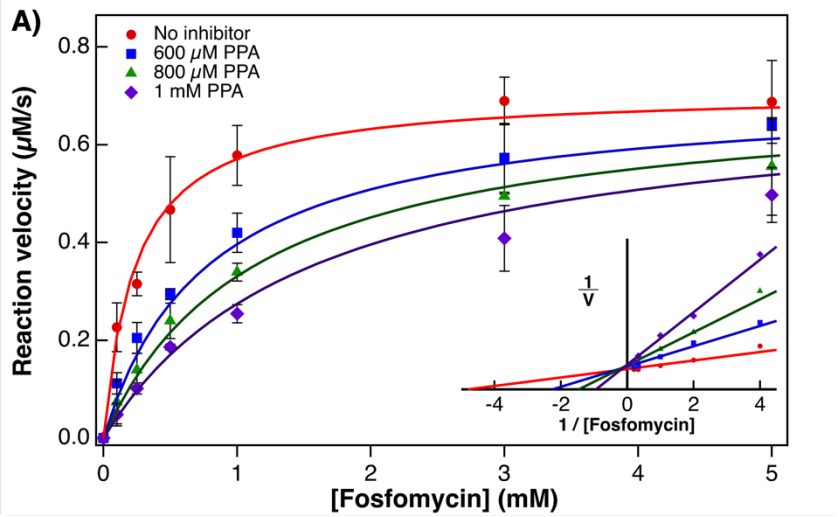


Figure S9. Glide docking output for each of the compounds in our working library. Each image represents the docking pose corresponding to the Glide score given in Table 1 in the main text.



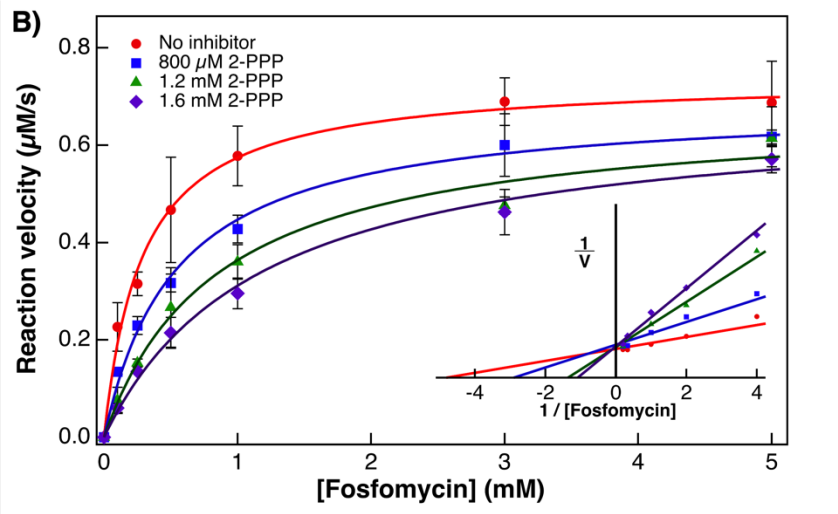


Figure S10. Michaelis-Menten kinetics of FosB^{Sa} inhibition. **A** shows inhibition by 600 μM, 800 μM, or 1 mM PPA. **B** shows inhibition by 800 μM, 1.2 mM, or 1.6 mM 2-PPP. Inset graphs show the Lineweaver-Burk plots of each.

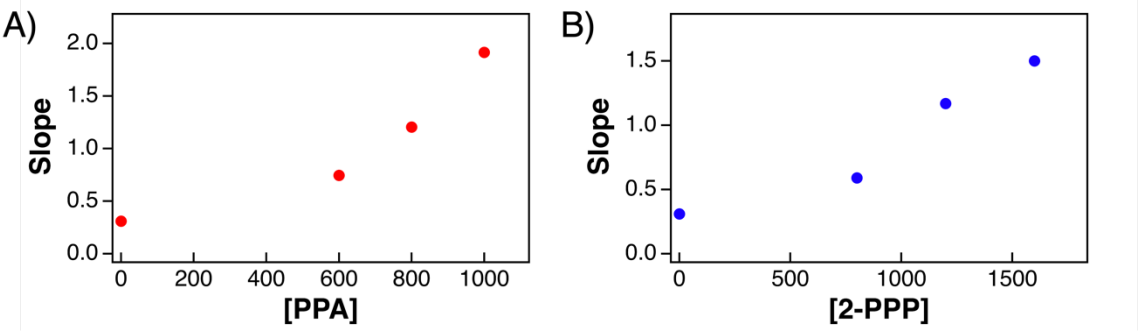


Figure S11. Replots of the slopes from the Lineweaver-Burk plots shown in Figure S10 with respect to inhibitor concentration. **A** shows the data for PPA. **B** shows the data for 2-PPP.

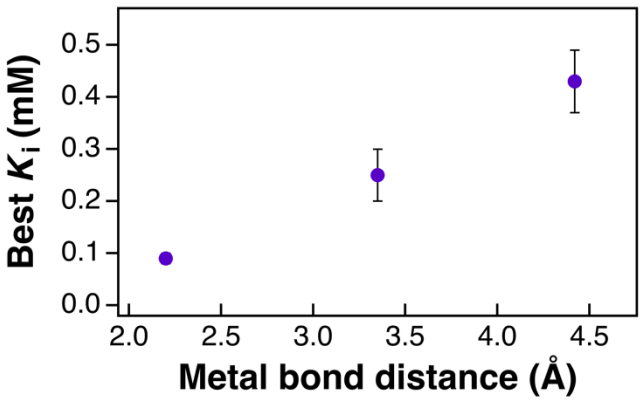
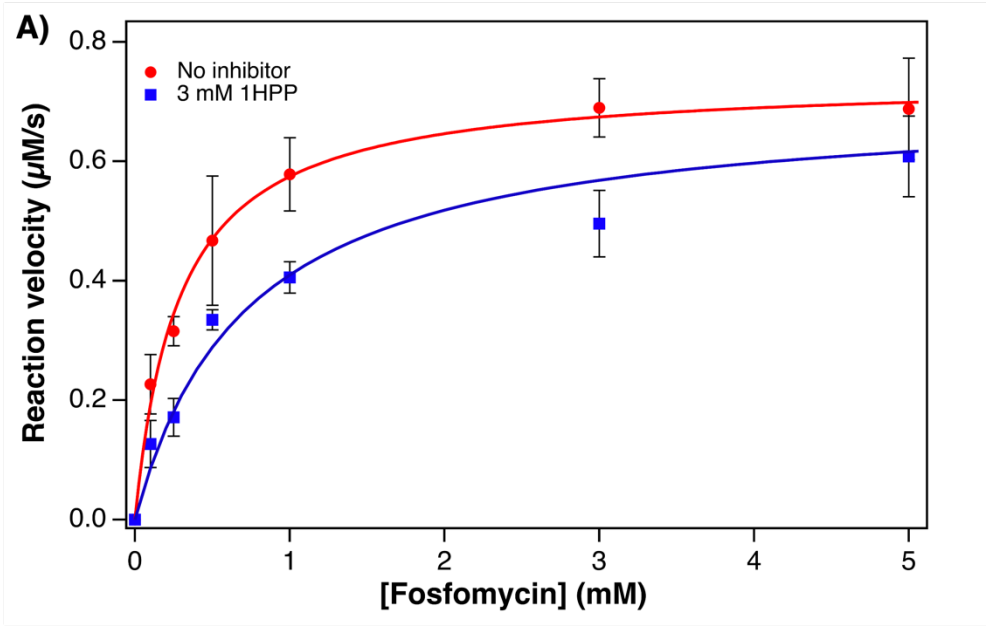
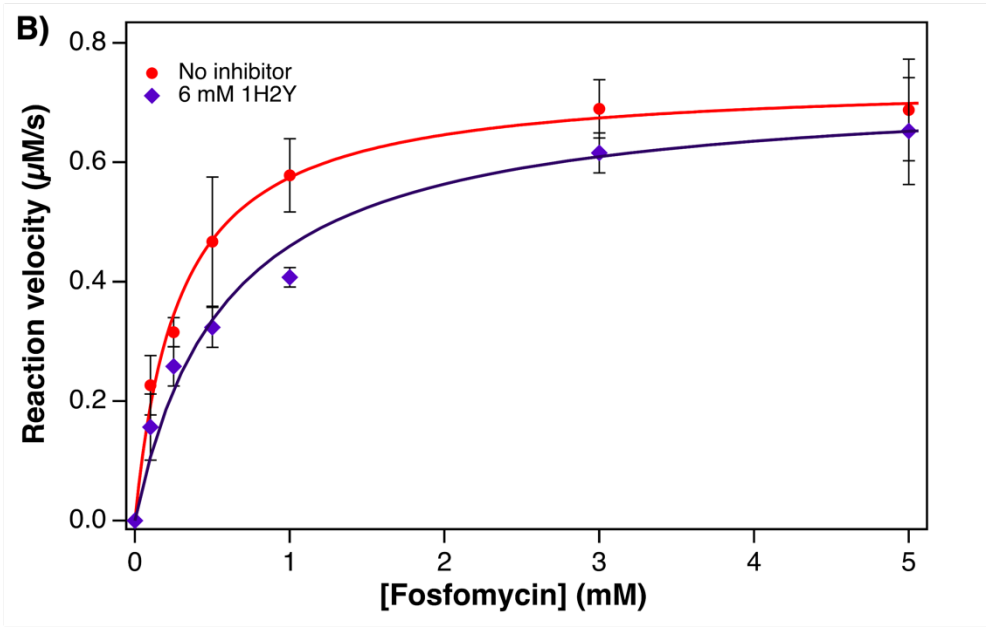


Figure S12. Plot of the best K_i values of PPF, compound **4**, and compound **5** compared to their carboxylate oxygen-metal distance.


A)
No inhibitor
3 mM 1HPP
Reaction velocity (μM/s)
0.0
0.2
0.4
0.6
0.8
0
1
2
3
4
5
[Fosfomycin] (mM)


B)
No inhibitor
6 mM 1H2Y
Reaction velocity (μM/s)
0.0
0.2
0.4
0.6
0.8
0
1
2
3
4
5
[Fosfomycin] (mM)

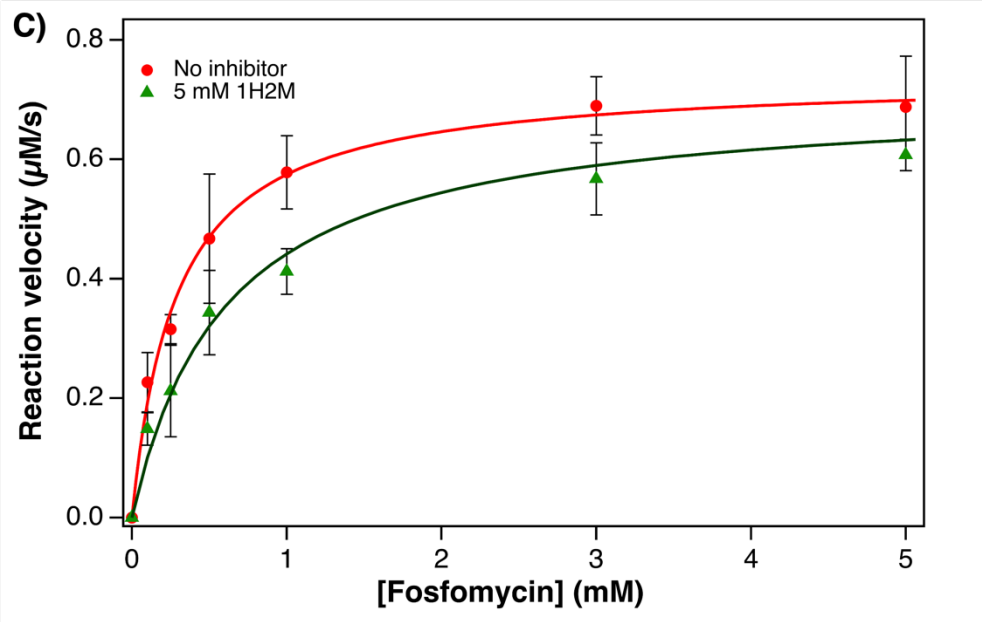


Figure S13. Michaelis-Menten kinetics of FosB^{Sa} inhibition with **A)** 3 mM 1HPP (compound **1**), **B)** 6 mM 1H2Y (compound **2**), or **C)** 5 mM 1H2M (compound **3**).

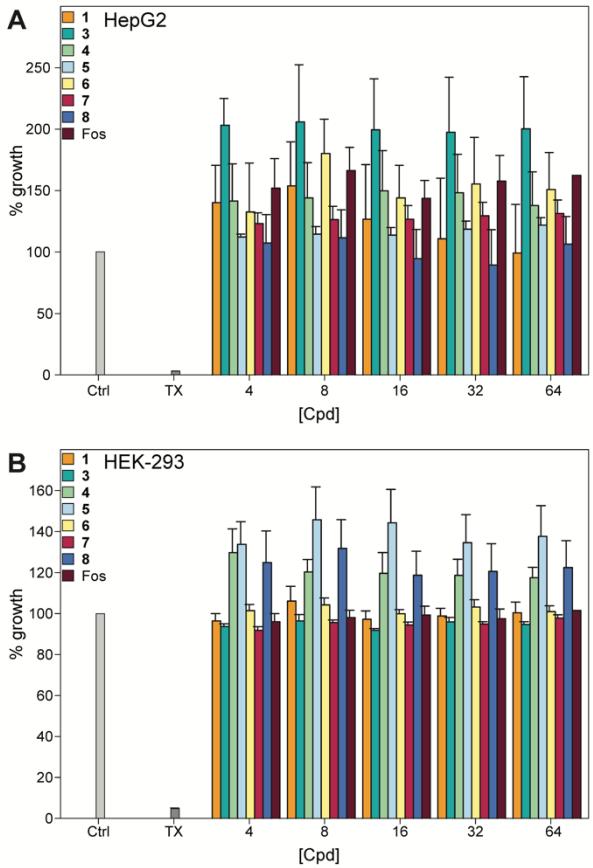


Figure S14. Mammalian cell cytotoxicity of compounds **1**, **3-8**, and fosfomycin at varying concentrations against HepG2 (top panel) and HEK-293 (bottom panel) cell lines. *Note*: The corresponding normalized plots can be found in the main text (Figure 5).

References

1. C. J. Williams, J. J. Headd, N. W. Moriarty, M. G. Prisant, L. L. Videau, L. N. Deis, V. Verma, D. A. Keedy, B. J. Hintze, V. B. Chen, S. Jain, S. M. Lewis, W. B. Arendall, 3rd, J. Snoeyink, P. D. Adams, S. C. Lovell, J. S. Richardson and D. C. Richardson, *Protein Sci*, 2018, **27**, 293-315.