# SUPPORTING INFORMATION

#### Small molecule inhibitors of Ca<sup>2+</sup>-S100B reveal two protein conformations

Michael C. Cavalier<sup>†</sup>, Mohd. Imran Ansari<sup>#</sup>, Adam D. Pierce<sup>†</sup>, Paul T. Wilder<sup>†§</sup>, Laura E. McKnight<sup>†</sup>, E. Prabhu Raman<sup>#</sup>, David B. Neau<sup>‡</sup>, Padmavani Bezawada<sup>#</sup>, Milad J. Alasady<sup>†</sup>, Thomas H. Charpentier<sup>†</sup>, Kristen M. Varney<sup>†</sup>, Eric A. Toth<sup>†§¥</sup>, Alexander D. MacKerell, Jr.<sup>#§</sup>, Andrew Coop<sup>#§</sup>, and David J. Weber<sup>†§\*</sup>

<sup>†</sup> Center for Biomolecular Therapeutics (CBT), Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland 21201, United States. <sup>§</sup>Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland 21201, United States.

<sup>#</sup>Computer Aided Drug Design Center, University of Maryland, School of Pharmacy, Baltimore, Maryland 21201, United States. <sup>¥</sup>Institute for Bioscience and Biotechnology Research, 9600 Gudelsky Drive Rockville, MD 20850, USA

<sup>‡</sup>NE-CAT, Cornell University, Argonne IL 60439, USA.

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\*To whom correspondence should be addressed; D.J.W., University of Maryland School of Medicine, Department of Biochemistry and Molecular Biology, Biomedical Research Facility Rm. 439, 108 N. Greene St., Baltimore, MD 21201. Phone (410) 706-4354, Fax (410) 706-0438, E-mail: dweber@som.umaryland.edu.

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Table S1. Calculated RMSD (Å) of Models from $C^{\alpha}$ of <u>1MHO</u>		
<sup>Ca</sup> S100B•5a	0.830 (0.307)	
<sup>Ca</sup> S100B•6b	0.829 (0.328)	
<sup>Ca</sup> S100B•17	0.215 (0.215)	0.360 (0.235)
The protein chains of the introduced models were aligned with the		
protein chain within $\underline{1MHO}^{46}$ . Numbers in parentheses are the		
calculated RMSD of only the globally conserved residues 1-84.		



**Figure S1. The crystal structure of S100B overlaid with compound 9a.** (A) Top scoring conformation predicted by AutoDock, (B) Representative conformations calculated by MC-SILCS, (C) SILCS FragMaps are shown at a cutoff of -1.2 kcal/mol. Nonpolar maps are shown in green and positively charged group maps in cyan. The positively charged group maps drive the placement of the basic alkyl groups in the MC-SILCS docking.



**Figures S2-5. Assigned 2D** <sup>1</sup>**H-**<sup>15</sup>**N HSQC NMR Spectra.** The binding of inhibitors to <sup>Ca</sup>S100B was assessed by monitoring perturbations of backbone <sup>1</sup>H–<sup>15</sup>N HSQC NMR experiments.









**Figure S6. Measurements of Chemical Shift Perturbations from Assigned 2D** <sup>1</sup>H-<sup>15</sup>N HSQC NMR spectra.</sup> The solid horizontal line is plotted at the mean perturbation (Hz) plus one standard deviation for each dataset.

Figures S7-29. HPLC-MS. Purity of all compounds was determined to be >95% by HPLC. Compound 16a



# Compound 11



#### Compound 10



# Compound 9b



# Compound 9a



#### Compound 8



# Compound 7d





# Compound 7b



#### Compound 7a



#### Compound 6d



#### Compound 6c



# Compound 6b



#### Compound 6a



#### Compound 5d





#### Compound 5b



#### Compound 5a



# Compound 4c



#### Compound 4b



#### Compound 4a







# Figures S30-48(below). <sup>1</sup>H and <sup>13</sup>C NMR spectra for synthesized compounds obtained using a 500 MHz Varian NMR Spectrometer.



<sup>1</sup>H and <sup>13</sup>C NMR of 5a



<sup>1</sup>H and <sup>13</sup>C

NMR of 5b



<sup>1</sup>H and <sup>13</sup>C NMR of 5c



<sup>1</sup>H and <sup>13</sup>C NMR of 5d



<sup>1</sup>H and <sup>13</sup>C NMR of 6a



<sup>1</sup>H and <sup>13</sup>C

NMR of 6b



<sup>1</sup>H and <sup>13</sup>C NMR of 6c



<sup>1</sup>H and <sup>13</sup>C NMR of 6d

![](_page_39_Figure_0.jpeg)

![](_page_39_Figure_1.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 7a

![](_page_40_Figure_0.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 7b

![](_page_41_Figure_0.jpeg)

# <sup>1</sup>H NMR of 7c

![](_page_42_Figure_0.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 7d

![](_page_43_Figure_0.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 8

![](_page_44_Figure_0.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 9a

![](_page_45_Figure_0.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 9b

![](_page_46_Figure_0.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 10

![](_page_47_Figure_0.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 11

![](_page_48_Figure_0.jpeg)

![](_page_48_Figure_1.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 16a

![](_page_49_Figure_0.jpeg)

<sup>1</sup>H and <sup>13</sup>C NMR of 16b

Table	Table S2. SMILES Documentation		
Drop	Internal		
riep.	I.D.	SMILE	
I.D.	SBiX		
4a	4211	N=C(N)C(C=C1)=CC=C1OCCCCCCCCC2=CC=C(C(N)=N)C=C2	
4b	1	N=C(C1=CC=C(OCCCCCOC2=CC=C(C(N)=N)C=C2)C=C1)N	
4c	4210	N=C(N)C(C=C1)=CC=C1OCCCCCCOC2=CC=C(C(N)=N)C=C2	
5a	4225	C1(C2=NCCN2)=CC=C(OCCCCCCCC3=CC=C(C4=NCCN4)C=C3)C=C1	
5b	4224	C1(C2=NCCN2)=CC=C(OCCCCCOC3=CC=C(C4=NCCN4)C=C3)C=C1	
5c	4226	C1(C2=CC=C(OCCCCCOC3=CC=C(C4=NCCN4)C=C3)C=C2)=NCCN1	
5d	4221	C1(C2=CC=C(OCCCCCCCCC3=CC=C(C4=NCCN4)C=C3)C=C2)=NCCN1	
6а	4213	C1(C2=NCCCN2)=CC=C(OCCCCCCCCC3=CC=C(C4=NCCCN4)C=C3)C=C1	
6b	4214	C1(C2=NCCCN2)=CC=C(OCCCCCOC3=CC=C(C4=NCCCN4)C=C3)C=C1	
6c	4218	C1(C2=NCCCN2)=CC=C(OCCCCCOC3=CC=C(C4=NCCCN4)C=C3)C=C1	
6d	4217	C1(C2=NCCCN2)=CC=C(OCCCCCCCCCC3=CC=C(C4=NCCCN4)C=C3)C=C1	
7a	4227	C1(C2=CC=C(OCCCCCCCC3=CC=C(C4=NCCCCCN4)C=C3)C=C2)=NCCCCCN1	
7b	4223	C1(C2=CC=C(OCCCCCOC3=CC=C(C4=NCCCCN4)C=C3)C=C2)=NCCCCN1	
7c	4228	C1(C2=CC=C(OCCCCCOC3=CC=C(C4=NCCCCN4)C=C3)C=C2)=NCCCCN1	
7d	4222	C1(C2=CC=C(OCCCCCCCCCC3=CC=C(C4=NCCCCN4)C=C3)C=C2)=NCCCCN1	
8	4236	N=C(NCCCCCN)C(C=C1)=CC=C1OCCCCCOC2=CC=C(C(NCCCCCN)=N)C=C2	
9a	4232	N=C(NCCCCCCN)C(C=C1)=CC=C1OCCCCCCOC2=CC=C(C(NCCCCCCN)=N)C=C2	
9b	4230	N=C(NCCCCCCN)C1=CC=C(OCCCCCCCCCC2=CC=C(C(NCCCCCCCN)=N)C=C2)C=C1	
10	4235	N=C(NC(NC1=CC=CC=C1)=O)C(C=C2)=CC=C2OCCCCCCCCCCCC3=CC=C(C(NC(NC4=CC=CC=C4)=O)=N)	
		C=C3	
11	4212	CNC(NC(C1=CC=C(OCCCCCCCCC2=CC=C(C(NC(NC)=O)=N)C=C2)C=C1)=N)=O	
16a	4239	NC(C(C=C1)=CC=C1OCCCN(CC2=CC=CC=C2)CCCOC3=CC=C(C(N)=N)C=C3)=N	
16b	4238	NC(C(C=C1)=CC=C1OCCCN(CCC)CCCOC2=CC=C(C(N)=N)C=C2)=N	
17	29	N=C(C1=CC=C(OC2=CC=C(C3=CC(C=CC(C(N)=N)=C4)=C4N3)C=C2)C=C1)N	