

Supporting Information for

In silico identification of novel inhibitors targeting the homodimeric interface of superoxide dismutase from the dental pathogen *Streptococcus mutans*

Carmen Cerchia ^{1,†}, Emanuela Roscetto ^{2,†}, Rosarita Nasso ^{2,3}, Maria Rosaria Catania ², Emmanuele De Vendittis ², Antonio Lavecchia ¹, Mariorosario Masullo ^{2,3,4,*} and Rosario Rullo ^{2,5,*}

¹ Department of Pharmacy, "Drug Discovery" Laboratory, University of Naples Federico II, 80131 Naples, Italy; carmen.cerchia@unina.it (C.C.); antonio.lavecchia@unina.it (A.L.)

² Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, 80131 Naples, Italy; emanuela.roschetto@unina.it (E.R.); mariarosaria.catania@unina.it (M.R.C.); devendit@unina.it (E.D.V.)

³ Department of Human Movement Sciences and Wellness, University of Naples "Parthenope", 80133 Naples, Italy; rosarita.nasso@collaboratore.uniparthenope.it (R.N.); mario.masullo@uniparthenope.it (M.M.)

⁴ CEINGE, Biotecnologie Avanzate S.C.a R.L., 80145 Naples, Italy

⁵ Institute for the Animal Production Systems in the Mediterranean Environment, 80147 Naples, Italy; rosario.rullo@cnr.it (R.R.)

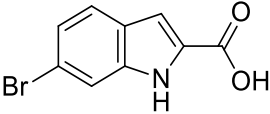
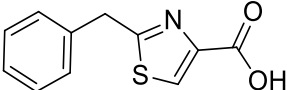
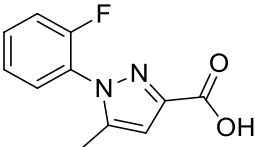
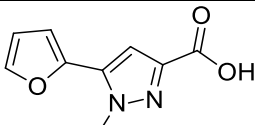
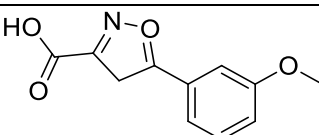
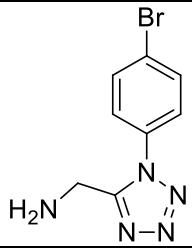
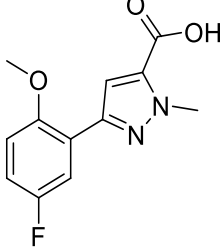
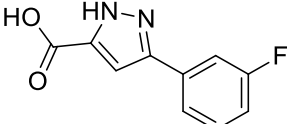
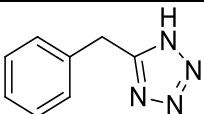
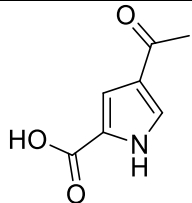
Table of Contents

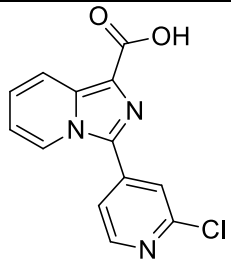
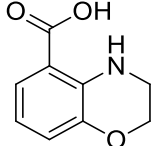
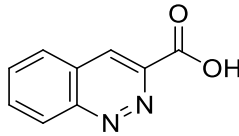
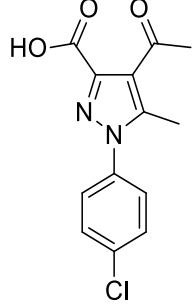
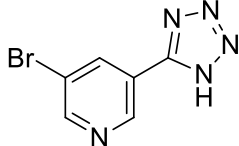
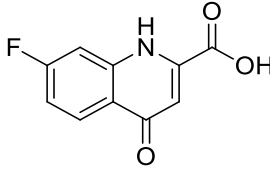
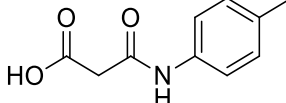
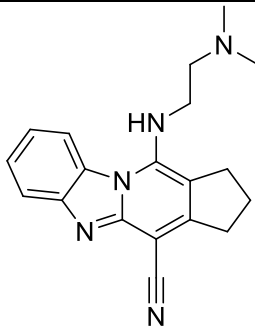
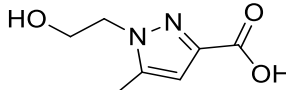
- Figure S1. Sequence alignments of mature forms of *Sm*SOD, *St*SOD, Fe-SOD from *E. coli* and Mn-SOD from rat mitochondria
- Table S1. Compounds identified by virtual screening
- Table S2. Values of IC₅₀ obtained for various SODs in the presence of **ALS-31**

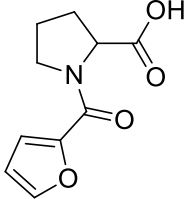
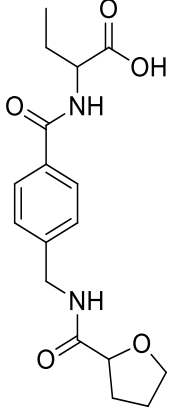
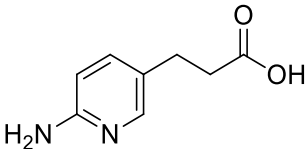
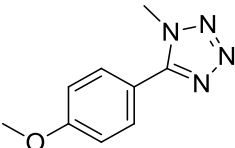
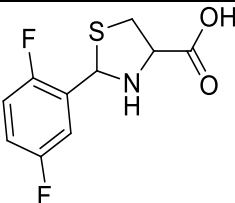
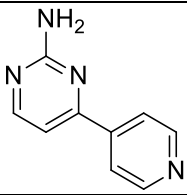
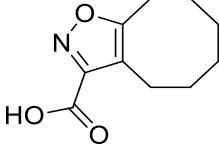
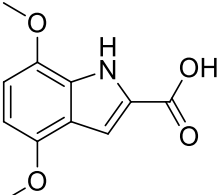
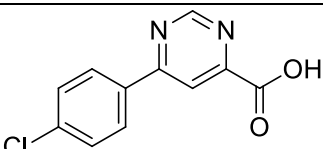
<i>Sm</i> SOD	AILLPDLPYA	YDALEPYIDA	ETMTLHHDKH	HATYVANANA	ALEKHPEIGE	NLEVLLADVE	60
<i>St</i> SOD	AIILPDLPYA	YDALEPYIDA	ETMTLHHDKH	HATYVANANA	ALEKHPEIGE	DLEALLADVE	
Fe-SOD (<i>E. coli</i>)	SFELPALPYA	KDALAPHISA	ETIEYHYGKH	HQTYVTNLNN	LIKGTAFEGK	SLEEIIRSSE	
Mn-SOD (rat)	KHSLPDLPYD	YGALEPHINA	QIMQLHHSKH	HATYVNNLNV	TEEKYH----	---EALAKGDV	
	** ***	.** *.*.*	: : *.*.*	* *** * *	:	* : .	
<i>Sm</i> SOD	QIPADIRQSL	INNNGGHLNH	ALFWELLSPE	KT-KVTAEVA	AAINEAFGSF	DDFKAAFTAA	119
<i>St</i> SOD	KIPADIRQAL	INNNGGHLNH	ALFWELLSPE	KQ-EPTAEVA	AAINEAFGSF	EAFAQEVFTTS	
Fe-SOD (<i>E. coli</i>)	G-----GV	FNNAAQVWNH	TFYWNCLAPN	AGGEPTGKVA	EAIAASFGSF	ADFKAQFTDA	
Mn-SOD (rat)	TTQVALQPAL	KFNGGGHINH	SIFWTNLSPK	GGGEPKGELL	EAIKRDFGSF	EKFKEKLTAV	
	.: *.. **	:::* *:.*	: ...:	** ****	*: :*		
<i>Sm</i> SOD	ATTRFGSGWA	WLVDKE-GK	LEVTSTANQD	TPI--SQGLK	PILALDVWEH	AYYLNRYNVR	176
<i>St</i> SOD	ATTRFGSGWA	WLVDNAE-GK	LEVSTPNQD	TPI--SDGKK	PILALDVWEH	AYYLYKNVR	
Fe-SOD (<i>E. coli</i>)	AIKNFGSGWT	WLKNSD-GK	LAIVSTSNAG	TPL--TTDAT	PLLTVDVWEH	AYYIDYRNAR	
Mn-SOD (rat)	SVGQVQSGWG	WLGFNKEQGR	LQIAACSNQD	-PLQGTGLI	PLLGDVWEH	AYYLYKNVR	
	: **** **	: : *	* :.: .*	*: : .	*.* :*****	***:.*.*.*	
<i>Sm</i> SOD	PNYIKAFFEV	INWNTVARLY	AEALTK	202			
<i>St</i> SOD	PNYIKAFFEI	INWNKVAELY	AEA--K				
Fe-SOD (<i>E. coli</i>)	PGYLEHFWAL	VNWEFVAKNL	AA----				
Mn-SOD (rat)	PDYLKAIWNV	INWENVSQRY	IVC-KK				
	..: : : : *						

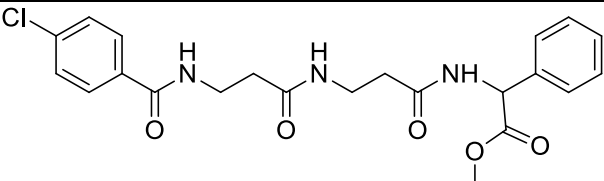
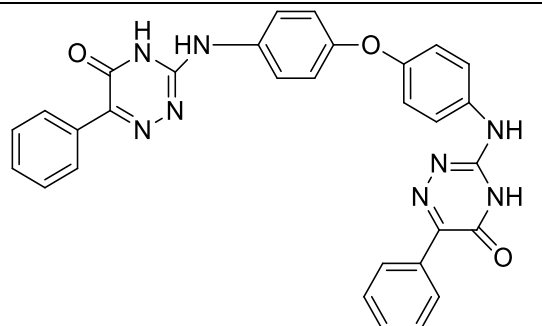
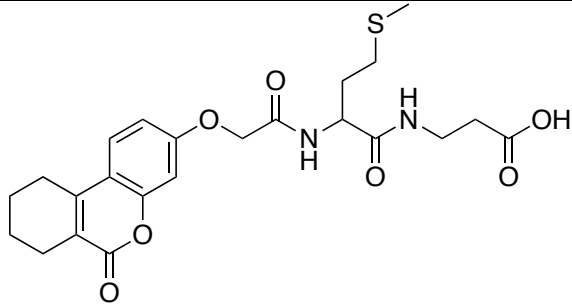
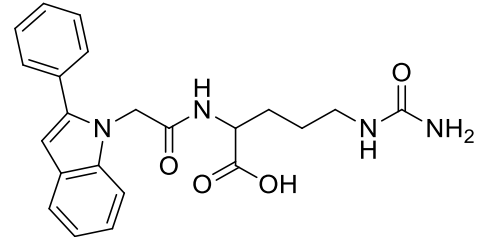
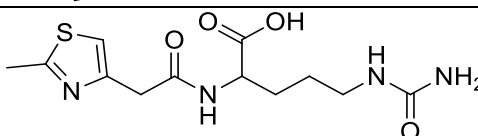
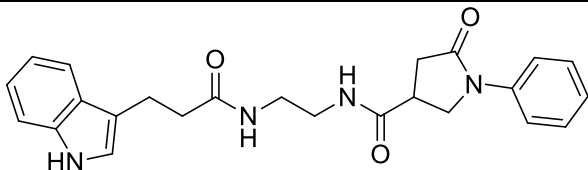
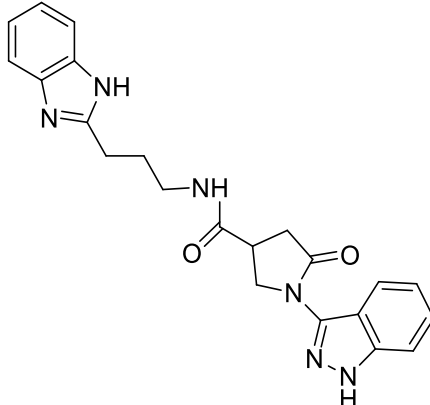
Figure S1. Sequence alignments of mature forms of *Sm*SOD, *St*SOD, Fe-SOD from *E. coli* and the Mn-SOD from rat mitochondria. Alignment was performed with MUSCLE [Edgar, R.C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797]. The residues mainly involved in dimer assembly (E21, H30, S126, E165, H166, Y169, R173, residue numbering referred to the PDB structure 4YIP) are highlighted in pink.

Table S1. Compounds identified by virtual screening.

N.	Code	Approach	ID	Structure
1	1149684	LBVS	ALS-1	
2	1088043	LBVS	ALS-2	
3	1794806	LBVS	ALS-3	
4	2251077	LBVS	ALS-4	
5	1593018	LBVS	ALS-5	
6	2201826	LBVS	ALS-6	
7	3543934	LBVS	ALS-7	
8	5042299	LBVS	ALS-8	
9	1973472	LBVS	ALS-9	
10	2229874	LBVS	ALS-10	

11	9080680	LBVS	ALS-11	
12	4041753	LBVS	ALS-12	
13	1780792	LBVS	ALS-13	
14	6328802	LBVS	ALS-14	
15	7119870050	LBVS	ALS-15	
16	3339531	LBVS	ALS-16	
17	1975653	LBVS	ALS-17	
18	0128660069	SBVS	ALS-18	
19	1086703	LBVS	ALS-19	

20	1045880	LBVS	ALS-20	
21	1331803	SBVS	ALS-21	
22	6923424	LBVS	ALS-22	
23	6051822	LBVS	ALS-23	
24	7138831	LBVS	ALS-24	
25	2222682	LBVS	ALS-25	
26	2214908	LBVS	ALS-26	
27	2270454	LBVS	ALS-27	
28	3533961	LBVS	ALS-28	

29	2195404	SBVS	ALS-29	
30	4794984	SBVS	ALS-30	
31	1098697	SBVS	ALS-31	
32	3460720	SBVS	ALS-32	
33	6242859	SBVS	ALS-33	
34	6244314	SBVS	ALS-34	
35	7131627	SBVS	ALS-35	

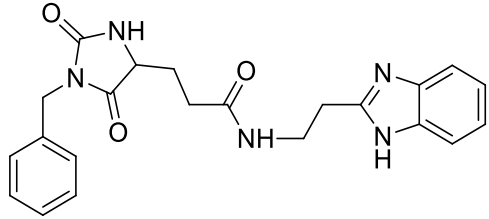
36	7567692	SBVS	ALS-36	 <p>The chemical structure of ALS-36 is a complex molecule featuring a benzimidazole ring system on the right, connected via a methylene group to a carbonyl group. This carbonyl is part of a chain that includes a hydantoin-like moiety (a five-membered ring with two carbonyl groups and an NH group) which is further substituted with a benzyl group. The full structure is: <chem>c1ccc2c(c1)c(c[nH]2)CCNC(=O)CCC(=O)Nc3ccccc3</chem></p>
----	---------	------	--------	---

Table S2. Values of IC₅₀ obtained for various SODs in the presence of **ALS-31**

SOD group	Source	IC ₅₀ (μM)
Cambialistic	<i>Streptococcus mutans</i>	159 ± 19
"	<i>Streptococcus thermophilus</i>	218 ± 50
Fe-SOD	<i>Escherichia coli</i>	123 ± 20
Mn-SOD	Rat mitochondria	161 ± 56
Cu/Zn-SOD	Bovine erythrocytes	146 ± 25