Supplemental Material to

δ^1-Pyrroline-5-carboxylate reductase as a new target for therapeutics: inhibition of the enzyme from *Streptococcus pyogenes* and effects *in vivo*

Amino acids

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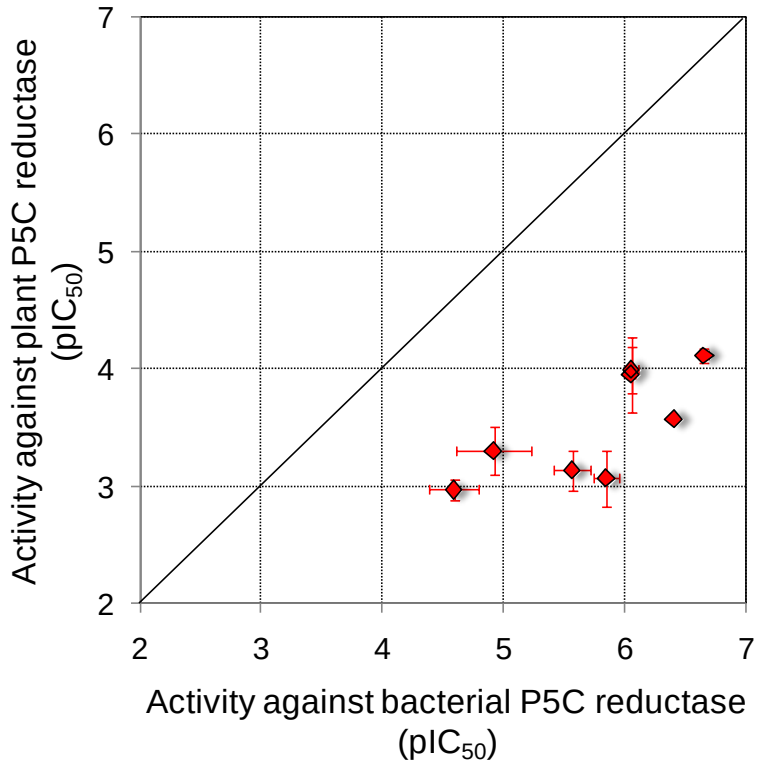


Fig. S1. Comparison between the effectiveness of bisphosphonates 08, 09, 11, 12, 13, 14, 15, and 16 against the bacterial and the plant P5C reductases. The inhibitory potential is expressed as the inverse of the logarithm of the concentration that 50%-inhibits the enzyme (pIC_{50}), ± SEM. The 1:1 line indicates equipotency. A point above the line shows that a given compound is less effective against the *S. pyogenes* P5C reductase, *vice versa* for a point below the line. Data for the plant enzyme are quoted from Forlani et al. (2008a).

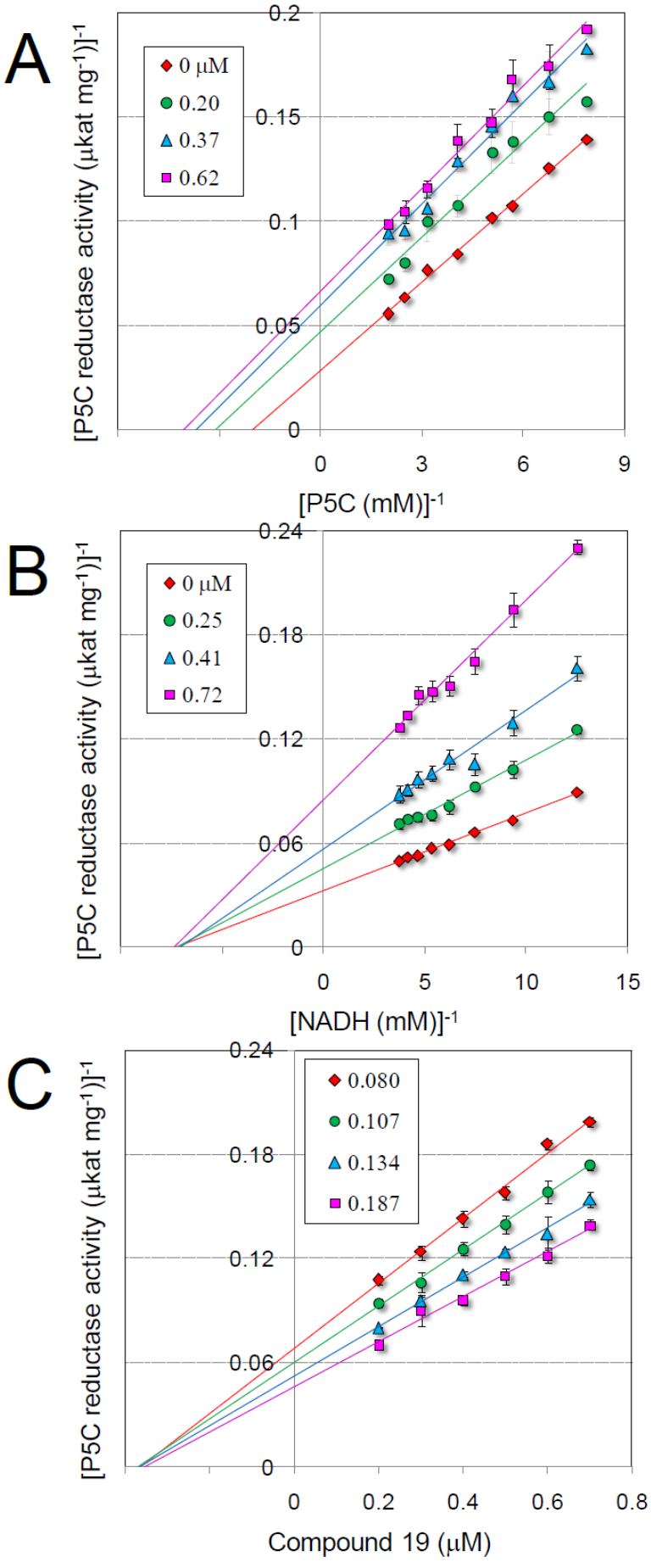
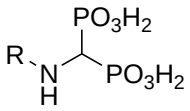
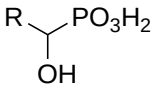
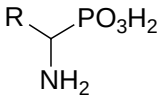
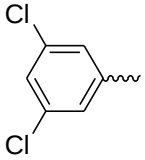
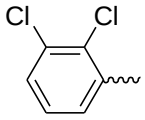
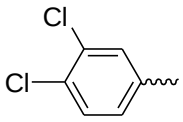
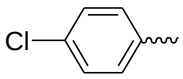


Fig. S2. Kinetic analysis of the inhibition of *S. pyogenes* P5C reductase by compound 19.

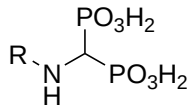
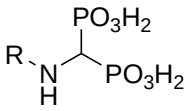
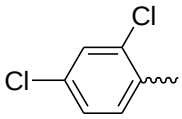
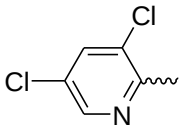
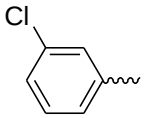
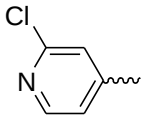
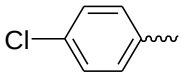
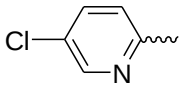
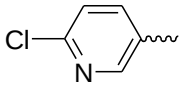
The enzyme was incubated at varying P5C (panel A) or NADH (panel B) level in the presence of increasing inhibitor concentrations, as indicated. Unvariable substrate concentration was fixed at 1 mM, 0.4 mM and 0.2 mM for L-P5C, NADH and NADPH, respectively. Parallel lines and lines converging to the x-axis accounted for an inhibition of uncompetitive and non competitive type, respectively. A Dixon plot of data obtained by assaying P5C reductase in the presence of increasing substrate concentration, as indicated, at varying inhibitor level (panel C) confirmed the non competitive mode of inhibition against NADH, and allowed K_I calculation.

Table S1 Comparison between the inhibition brought about by some active aminomethylene-bisphosphonates and that of the corresponding amino- and hydroxyphosphonates

			
R	Enzyme inhibition *		
	0.1 mM 0.2 mM	87.2 ± 0.8 % 91.1 ± 0.3 %	0.7 ± 1.4 % 4.7 ± 3.2 %	ND
	0.1 mM 0.2 mM	91.6 ± 0.9 % 95.4 ± 1.3 %	10.9 ± 0.8 % 23.3 ± 1.0 %	ND
	0.1 mM 0.2 mM	88.7 ± 0.7 % 92.5 ± 0.5 %	18.9 ± 1.1 % 37.6 ± 5.4 %	ND
	0.1 mM 0.2 mM	43.2 ± 1.4 % 61.5 ± 1.1 %	3.7 ± 1.2 % 6.9 ± 2.6 %	2.6 ± 0.7 % -5.9 ± 4.7 %

* P5C-dependent NADH oxidation was measured at 37°C for 5 min in the presence of 0.2 mM of a given compound. Results were expressed as percent of the activity measured in parallel, untreated controls. At least three replications were run for each phosphonate, and six for the control. Data are presented as percent inhibition, and are means ± SE over replications. ND, not determined. Bisphosphonates are achiral, whereas hydroxy- and aminophosphonates are racemic mixtures. However, the difference in the inhibitory activity largely exceeds that expected in the case of a racemic mixture in which one of the enantiomers is equivalent to the corresponding optically inactive bisphosphonate and the other enantiomer is inactive.

Table S2 Effect of replacing the phenyl substituent with a pyridyl moiety on the inhibitory potential of some active aminomethylenebisphosphonates.

 R =	Enzyme inhibition at 0.2 mM *	 R =	Enzyme inhibition at 0.2 mM *
	52.0 ± 1.2 %		10.2 ± 2.2 %
	71.2 ± 2.1 %		13.6 ± 7.0 %
	61.5 ± 1.1 %		-1.0 ± 2.5 %
			12.9 ± 2.5 %

* P5C-dependent NADH oxidation was measured at 37°C for 5 min in the presence of 0.2 mM of a given compound. Results were expressed as percent of the activity measured in parallel, untreated controls. At least three replications were run for each phosphonate, and six for the control. Data are presented as percent inhibition, and are means ± SE over replications.