



Phosphonic acid analogues of phenylglycine as inhibitors of aminopeptidases: Comparison of porcine aminopeptidase N, bovine leucine aminopeptidase and aminopeptidase from barley seeds

Weronika Wanat ¹, Michał Talma ¹, Małgorzata Pawełczak², and Paweł Kafarski ^{*1}

¹ Department of Bioorganic Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland;

² Faculty of Chemistry, University of Opole, ul. Oleska 48, 45-052, Opole, Poland

* Correspondence: pawel.kafarski@pwr.edu.pl; Tel.: +48 71 320 36 82

Supporting Information

Tables indicating the interactions between phosphonic acid analogues of phenylglycine and porcine aminopeptidase, bovine lens leucine aminopeptidase and tomato acidic leucine aminopeptidase

Table S2. Interactions of both isomers of compounds **8-14** with amino acids of porcine aminopeptidase (pAPN) taken for refinement during the IFD.

All	8R	8S	9R	9S	10R	10S	11R	11S	12R	12S	13R	13S	14R	14S
Gln206														
Gln208				Gln208			Gln208					Gln208		Gln208
Phe344				Phe344			Phe344					Phe344	Phe344	Phe344
Asn345													Asn345	
Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346	Ala346
Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347	Gly347
Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348	Ala348
Met349	Met349	Met349	Met349	Met349	Met349	Met349	Met349	Met349	Met349	Met349	Met349	Met349	Met349	Met349
Glu350	Glu350	Glu350	Glu350	Glu350	Glu350	Glu350	Glu350	Glu350	Glu350	Glu350	Glu350	Glu350		Glu350
Tyr357					Tyr357		Tyr357		Tyr357					Tyr357
Arg358		Arg358				Arg358		Arg358		Arg358				Arg358
Asn360														Asn360
Ala361		Ala361						Ala361		Ala361				Ala361
Arg376	Arg376	Arg376	Arg376		Arg376	Arg376		Arg376	Arg376	Arg376	Arg376			Arg376
Thr379	Thr379		Thr379	Thr379			Thr379		Thr379			Thr379		Thr379
Val380	Val380	Val380	Val380	Val380	Val380	Val380	Val380	Val380	Val380	Val380	Val380	Val380	Val380	Val380
Ile381							Ile381							
His383	His383	His383	His383	His383	His383	His383	His383	His383	His383	His383	His383	His383	His383	His383
Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384	Glu384
His387	His387	His387	His387	His387	His387	His387	His387	His387	His387	His387	His387	His387		His387
Glu406	Glu406	Glu406	Glu406	Glu406	Glu406	Glu406	Glu406	Glu406	Glu406	Glu406	Glu406	Glu406		Glu406
Ala409		Ala409	Ala409			Ala409		Ala409	Ala409	Ala409	Ala409			
Ser410	Ser410	Ser410	Ser410	Ser410	Ser410	Ser410	Ser410	Ser410	Ser410	Ser410	Ser410	Ser410		Ser410
Glu413	Glu413	Glu413	Glu413	Glu413	Glu413	Glu413	Glu413	Glu413	Glu413	Glu413	Glu413	Glu413		Glu413
Tyr414									Tyr414					
Gly433									Gly433					
Asp434			Asp434						Asp434		Asp434			
Arg437			Arg437						Arg437					
Phe467	Phe467	Phe467		Phe467	Phe467	Phe467	Phe467					Phe467		Phe467
Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472	Tyr472
Ser473			Ser473						Ser473		Ser473			
Zn1024	Zn1024	Zn1024	Zn1024	Zn1024	Zn1024	Zn1024	Zn1024	Zn1024	Zn1024	Zn1024	Zn1024	Zn1024		Zn1024

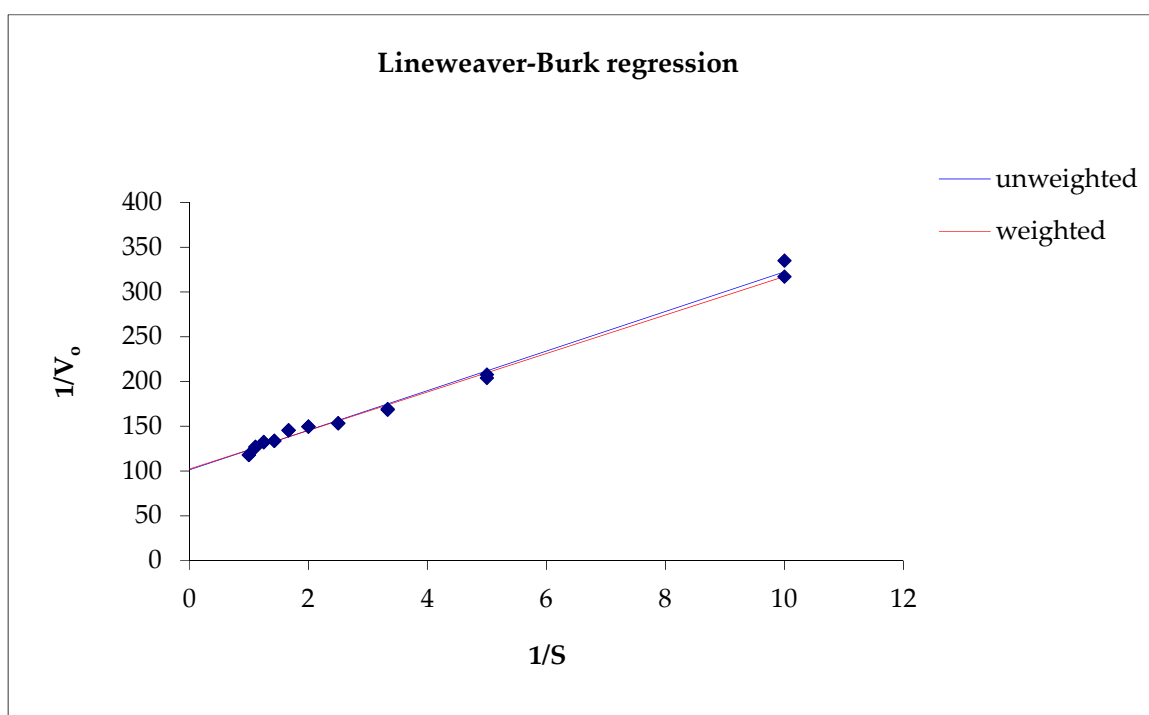
Enzyme assays

The progress of the enzymatic reactions was monitored spectrophotometrically (UV-VIS Spectrophotometer JASCO V-730) by following the change in absorbance at 405 nm (formation of *p*-nitroanilide). The extinction coefficient for *L*-Leu-*p*Na was $9620 \text{ M}^{-1}\text{cm}^{-1}$.

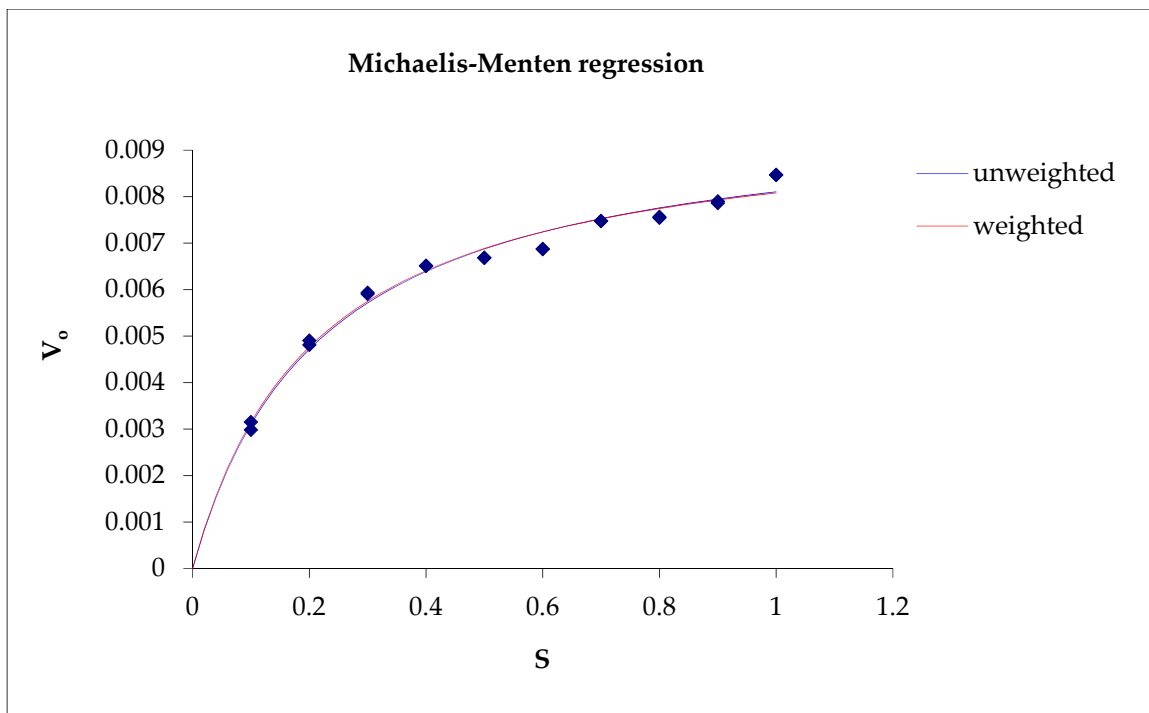
The kinetic parameters (K_M and V_{\max}) for aminopeptidase from barley seeds were determined by using the Lineweaver–Burk (L-B) weighted regression method. The measured K_M value was $0.21 \text{ mM} \pm 0.14$ and was used to determine the inhibitory constants of the all evaluated inhibitors.

Table S7. The kinetic parameters characterizing activity of the isolated aminopeptidase from barley seeds.

Metoda	K_M	S_{K_M}	V_{\max}	$S_{V_{\max}}$	R^2	a	b
L-B unweighted	0,218533	0,008905	0,009876	0,000186102	0,981321	1,001484	-2,7E-06
L-B weighted	0,210435	0,014394	0,009782	0,000190483	0,981347	0,985662	0,000112



Plot S1. Graphical determination of the K_M value for aminopeptidase from barley seeds by using Lineweaver-Burk: unweighted (blue line) and weighted (red line) regression method.



Plot S2. Graphical determination of the K_M value for aminopeptidase from barley seeds by using Michaelis-Menten: unweighted (blue line) and weighted (red line) non-linear regression method.

The value of K_M for porcine aminopeptidase (pAPN) was 0.52mM and was determined as described above.

