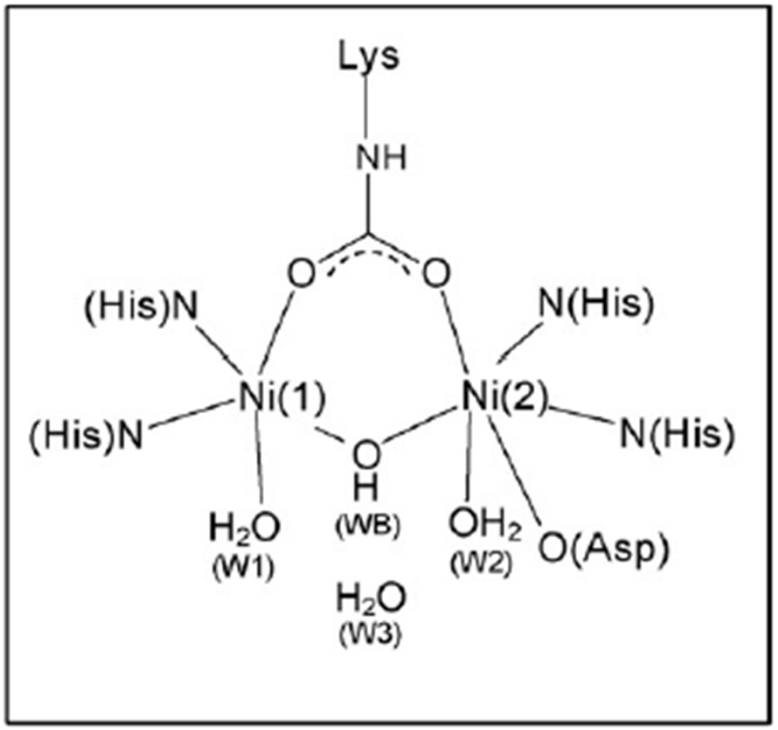
Wrocław University of Technology

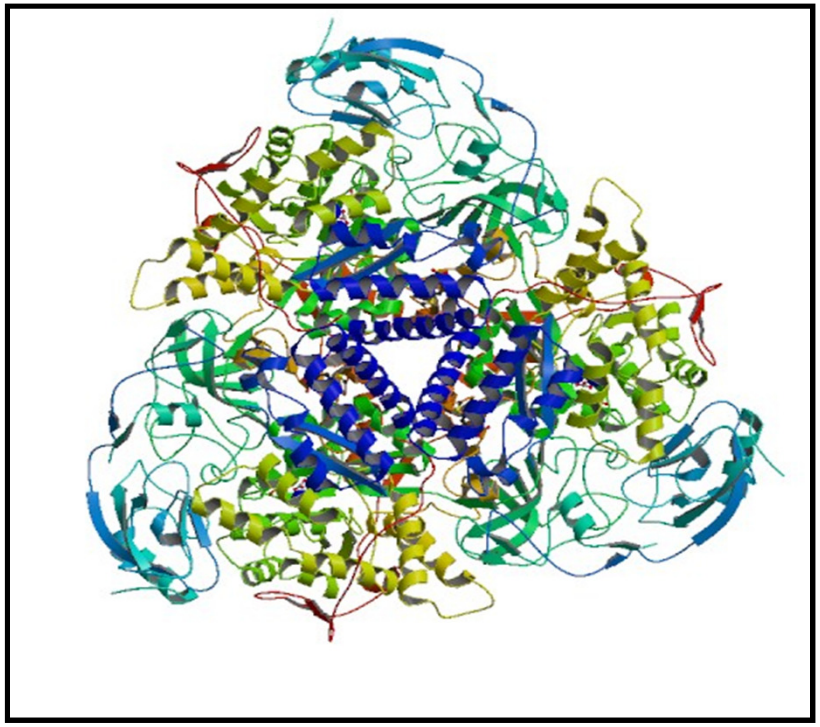
Novel urease inhibitors for the control of ureolytic microbial pathogens

Agnieszka Grabowiecka, Ewa Grela, Katarzyna Macegoniuk, Monika Biernat, Łukasz Berlicki, Artur Mucha, Paweł Kafarski

Urease - urea amidohydrolase 3.5.1.5

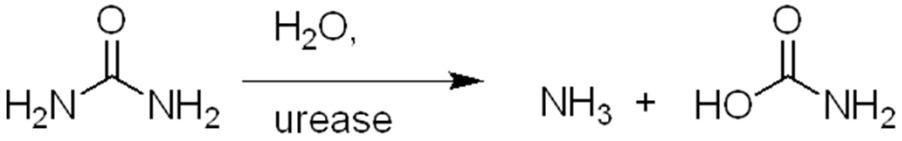


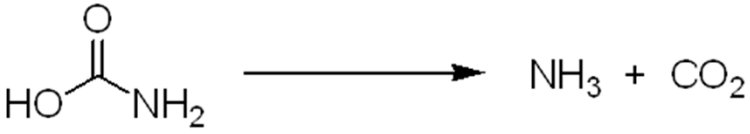
Benini, S., et al. Structure. 1999. 7 (2): p.205-216



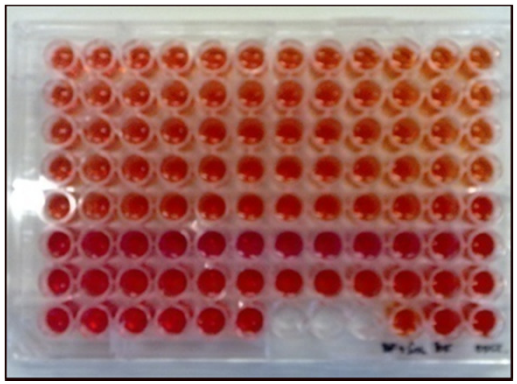
Bacillus pasteurii, PDBid 1S3T

Urease - reaction





$2NH_3 + 2H_2O \rightleftharpoons 2NH_4^+ + 2OH^-$



$(k_{cat}/K_m)/k_{uncat}$ $8 \cdot 10^{17}\ M^{-1}$

Regulation of microbial urease

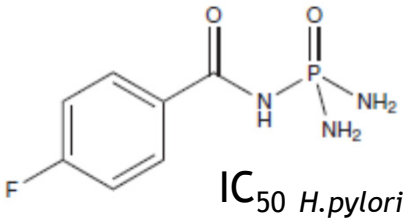
Mechanism	Microorganism
Urea induced	*Proteus mirabilis, Proteus vulgaris, Proteus penneri, Providencia rettgeri , Providencia stuartii*
Constitutive	*Sporosarcina pasteurii, Morganella morganii , Corynebacterium renale, Agrobacterium tumefaciens, Neurospora crassa, Rhizobium leguminosarum*
pH	*Streptococcus salivarius*
Repressive	*Klebsiella aerogenes, Pseudomonas fluorescens, Pseudomonas aeruginosa, Alcaligenes eutrophus, Bacillus megaterium , Micrococcus cerificans*

Inhibitors for the control of urease

Amides and esters of phosphoric and thiophosphoric acids

Enzymatic reaction transition state

transition state analogue



IC_{50} *H.pylori* 1.2 nM

Urea derivatives

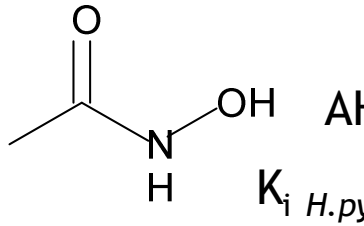
Heavy metal ions

Quinones

Polyphenols

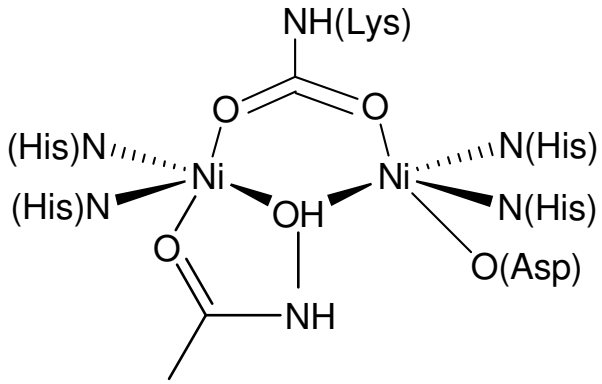
Heterocycles

Hydroxamic acids



AHA

K_i *H.pylori* 2μM



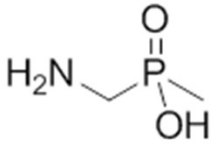
Benini, S.; Rypniewski, W.R.; Wilson, K.S.; Miletti, S.; Ciurli, S.; Mangani, S. The complex of Bacillus pasteurii urease with acetohydroxamate anion from X-ray data at 1.55 A resolution. *J. Biol. Inorg. Chem.* 2000, *5*, 110 - 118

Aminophosphinic analogs of phosphoramidates

transition state of enzymatic reaction

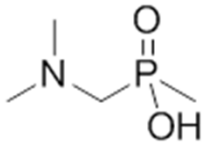
1

classical analogue of transition state

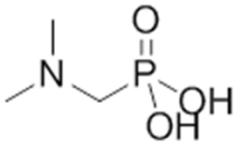


2

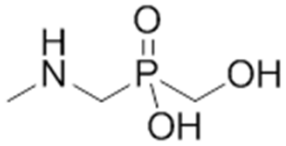
extended analogue of transition state



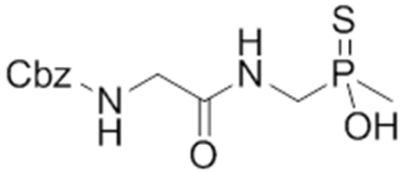
3, K_i = 0.62 μM



4, K_i = 13 μM



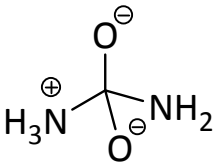
5, K_i = 0.43 μM

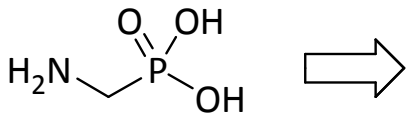


6, K_i = 0.17 μM

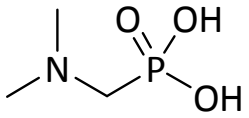
Stamatia Vassiliou, Agnieszka Grabowiecka, Paulina Kosikowska,Athanasios Yiotakis,Paweł Kafarski, and Łukasz Berlicki. Design, Synthesis, and Evaluation of Novel Organophosphorus Inhibitors of Bacterial Ureases. J. Med. Chem. 2008, 51, 5736-5744

Modification of P- and N- termini

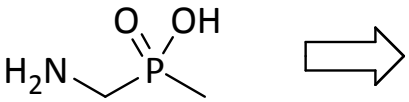




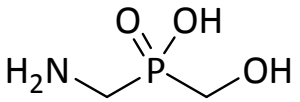
K_i = 314 µM (SPU)



K_i = 13 µM (SPU)



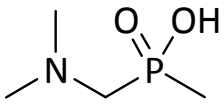
K_i = 340 µM (SPU)



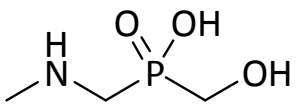
K_i = 95 µM (SPU)

Stamatia Vassiliou,Paulina Kosikowska,Agnieszka Grabowiecka, Athanasios Yiotakis, Pawe ł Kafarski, and Łukasz Berlicki.
Computer-Aided Optimization of Phosphinic Inhibitors of Bacterial Ureases.
J. Med. Chem. 2010, 53, 5597–5606

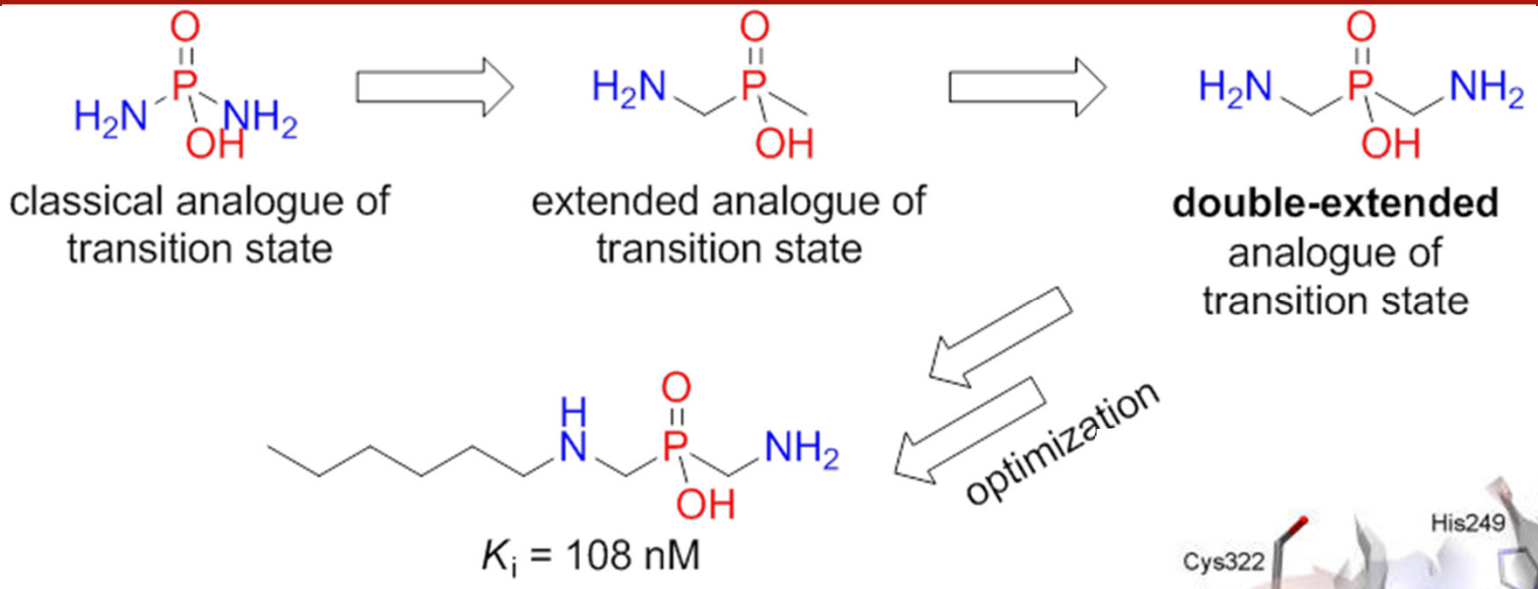
Łukasz Berlicki, Marta Bochno, Agnieszka Grabowiecka, Arkadiusz Białas, Paulina Kosikowska, Paweł Kafarski.
N-substituted aminomethanephosphonic and aminomethane-P-methylphosphinic acids as inhibitors of ureases.
Amino Acids (2012) 42:1937–1945

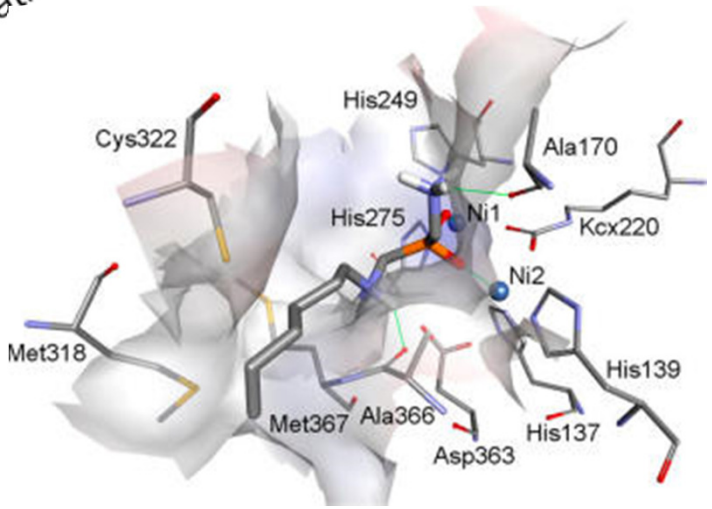


K_i = 0.62 µM (SPU)

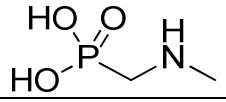
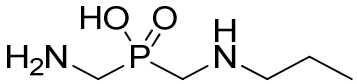
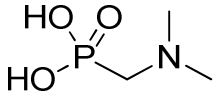
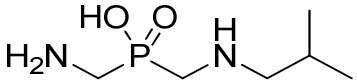
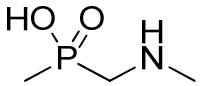
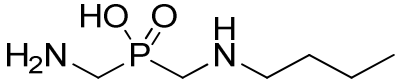
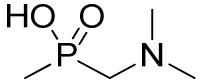
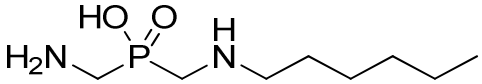
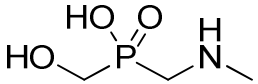
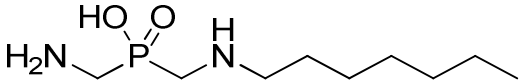
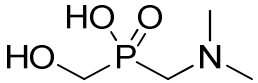
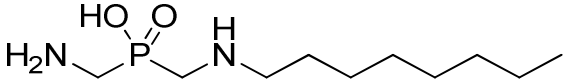
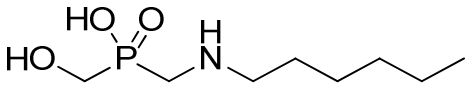
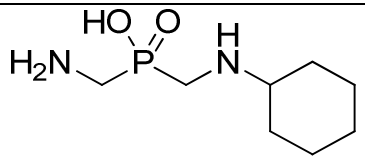
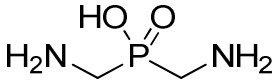
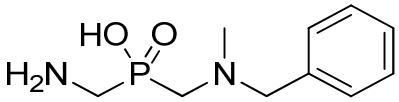
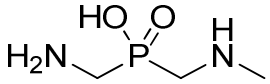
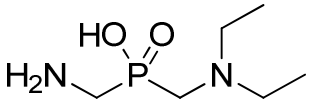
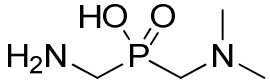
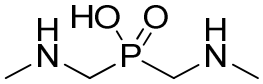
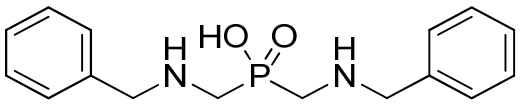


K_i = 0.43 µM (SPU)

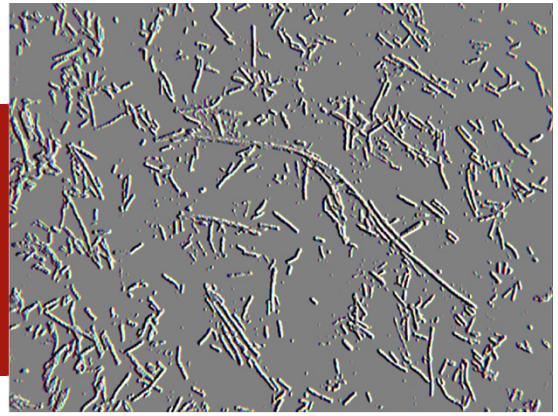




Katarzyna Macegoniuk, Anna Dziełak, Artur Mucha, and Łukasz Berlicki. ***Bis(aminomethyl)phosphinic Acid, a Highly Promising Scaffold for the Development of Bacterial Urease Inhibitors*****. ACS Med. Chem. Lett. 2015, 6, 146−150**

	Compound nr		
1		**11**	
2		**12**	
3		**13**	
4		**14**	
5		**15**	
6		**16**	
7		**17**	
8		**18**	
9		**19**	
10		**20**	
		21	

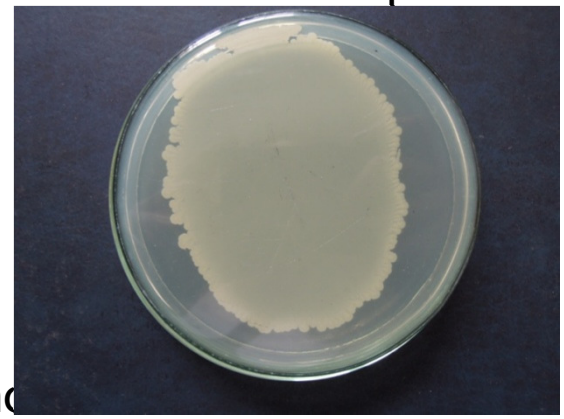
Proteus mirabilis



Adhesins and 17 fimbrial types – adhesion to uroepithelium and catheters

Swarming motility in rafts – polymicrobial ascending UTI

Agressive iron acquisition – unique proteobactin and enterobactins siderophores



Hemolysin and agglutinin toxins

Serralysin production

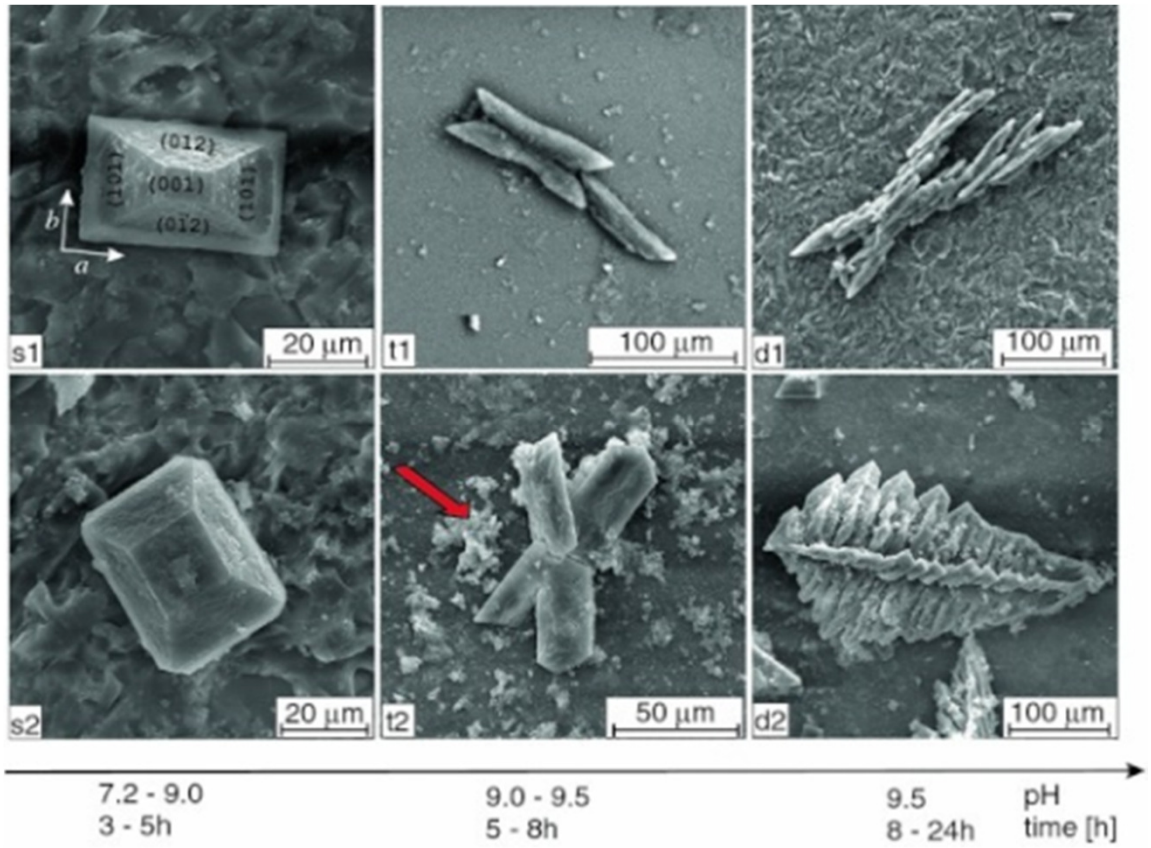
Biofilm formation

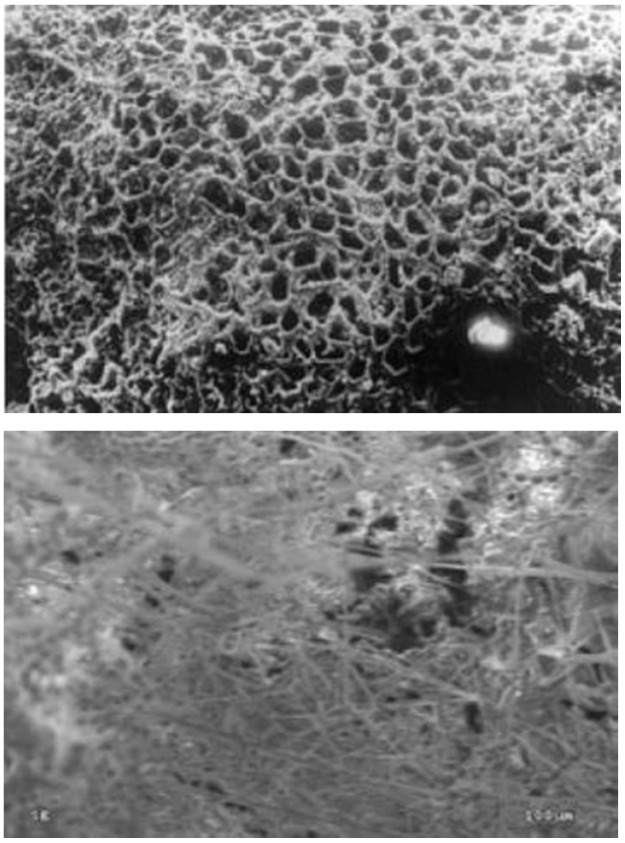
Horizontal gene transfer for pathogenicity and resistanc

Nitrogen metabolism – **urease** and glutamate dehydrogenase

Chelsie E. Armbruster and Harry L.T. Mobley. Merging mythology and morphology: the multiphaceted lifestyle of *Proteus Mirabilis*. *Nat. Rev. Microbiol*. 2012 10(11): 743-754

Crystallization in infected urine

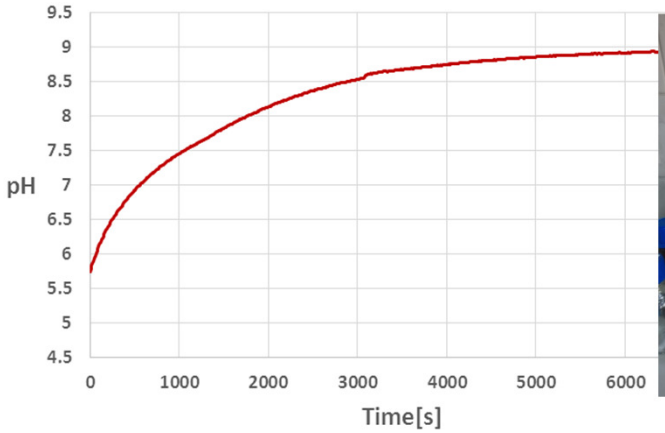


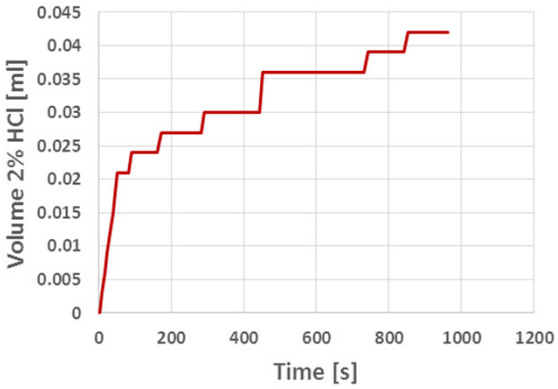


Unique surface and internal structure of struvite crystals formed by Proteus mirabilis Prywer J, Torzewska A, Płociński T - Urol. Res. (2012)

Przegląd Urologiczny 2010/2 (60)

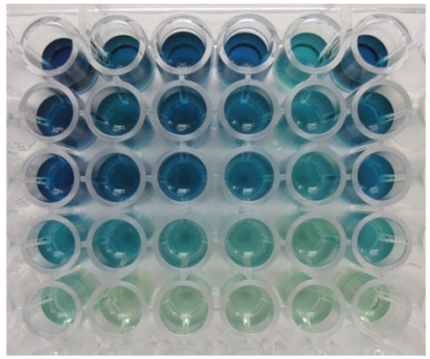
Proteus mirabilis PCM 543 – urease activity

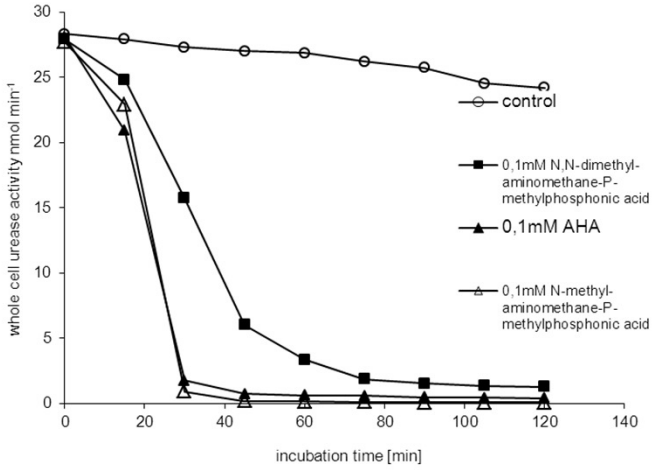




Indophenol blue assay

Colorimetric ammonium quantification

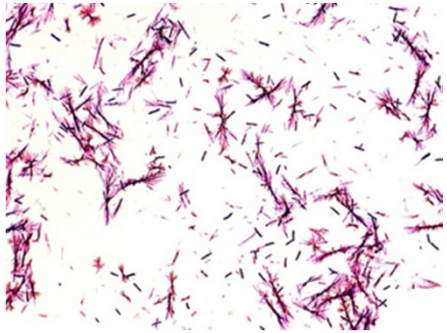


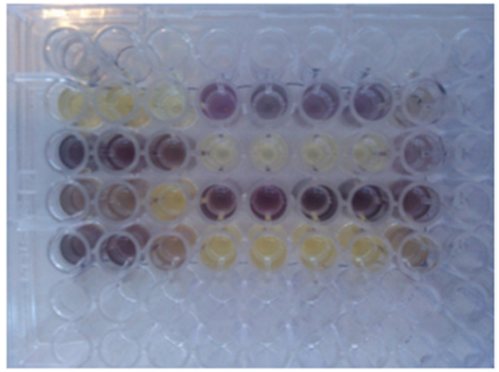


Proteus mirabilis PCM 543 – viability estimation

„MTT”

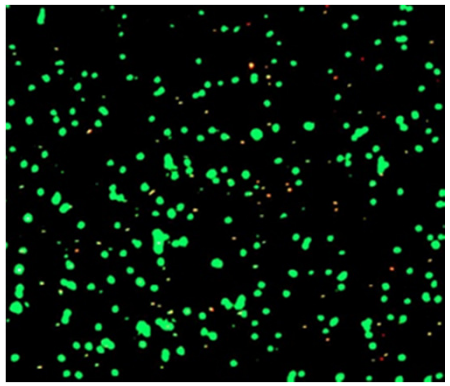
chromogenic assay of
dehydrogenase activity

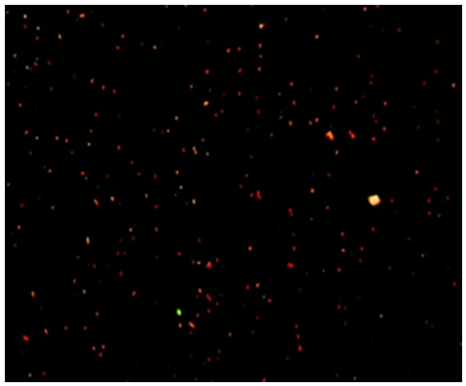




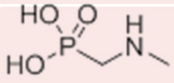
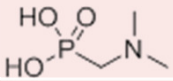
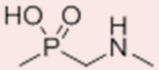
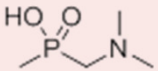
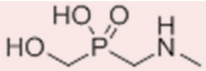
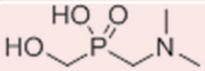
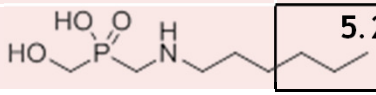
Fluorescent staining

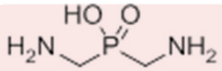
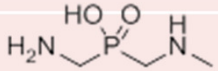
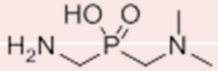
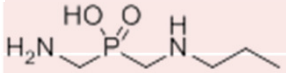
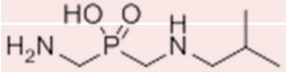
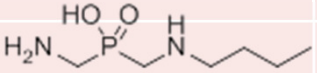
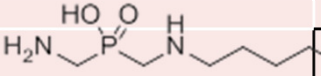
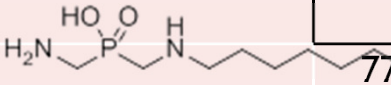
LIVE/DEAD BacLight L-7012

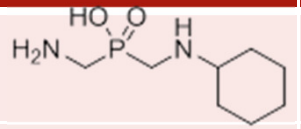
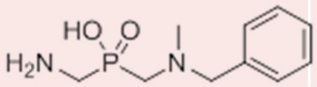
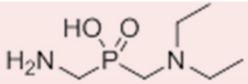
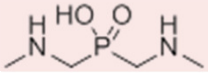
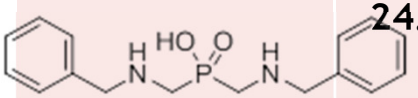




Influence of the studied urease inhibitors upon the whole-cell ureolytic activity and viability of *Proteus mirabilis* PCM543

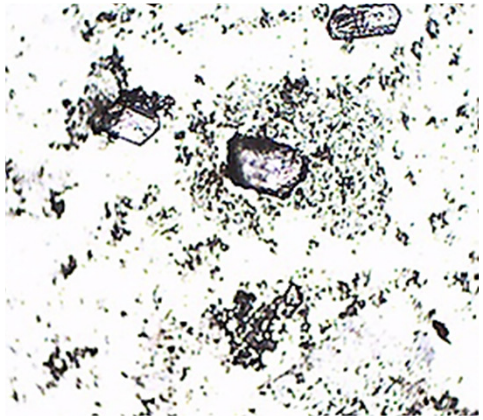
Compound	Ki [μM]	Percent preserved ureolytic activity	Percent Viability MTT	ΔNH_4^+ [mM]	ΔPO_4^{-3} [mM]	pH
Untreated culture		100	100	12	-6.5	9.6
	70±2.2	26±4	30±6	4.0	-3.4	7.4
	13±0.8	8±2	36±4	2.2	-0,6	5.6
	27±0.3	34±6	61±8	5.3	-0.29	7.5
	0.62±0.09	2±0.3	25±3	2.8	-1.4	6.0
	0.36±0.10	35±5	74±5	4.7	-3.7	7.4
	3.10±0.65	24±6	59±7	4.3	-1.8	7.2
	5.2±0.42	6±3	53±4	3.1	-1.6	6.1

Compound	Ki [μM]	Percent preserved ureolytic Activity	Percent Viability MTT	ΔNH_4^+ [mM]	ΔPO_4^{-3} [mM]	pH
	82 ±12	37±9	58±12	7.8	-4.6	8.2
	34 ± 8	10±2	63±6	3.3	-1.0	6.3
	24.4 ± 4.1	4±0.5	96±5	2.9	-1.3	6.15
	16.0 ± 1.1	17±5	57±9	3.6	-1.7	6.7
	416 ± 50	75±7	98±3	10.0	-5.8	9.2
	25.9 ± 3.0	32±8	65±5	11.5	-4.2	7.9
	0.202±0.057	4±0.7	40±7	2.5	-0.7	5.7
	778 ± 127	30±4	56±4	9.8	-5.1	8.6
	NI	68±11	60±6	9.0	-6.2	8.9

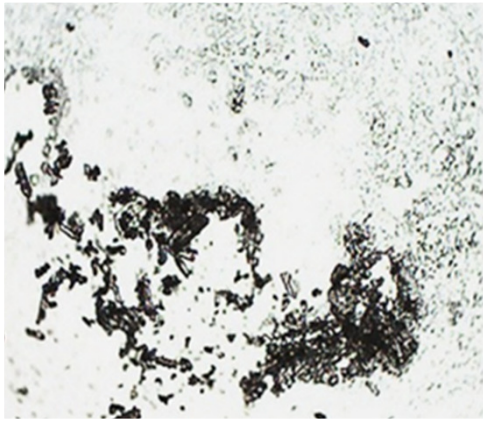
Compound	Ki [μM]	Percent preserved ureolytic Activity	Percent Viability MTT	ΔNH_4^+ [mM]	ΔPO_4^{-3} [mM]	pH
	NI	96±5	97±8	13.0	-6.3	9.5
	58.3 ± 8.0	92±6	90±7	7.0	-6.9	7.6
	NI	100	99±4	12.0	-6.6	8.5
	17.2± 2.5	26±7	76±7	4.5	-2.1	7.3
	24.52± 4.54	89±6	96±6	7.0	-6.7	8.4
AHA	5.7±4	42±6	56±7	9.2	-4.0	7.8

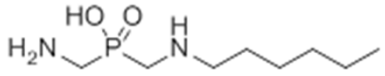
Urine precipitate

Untreated culture



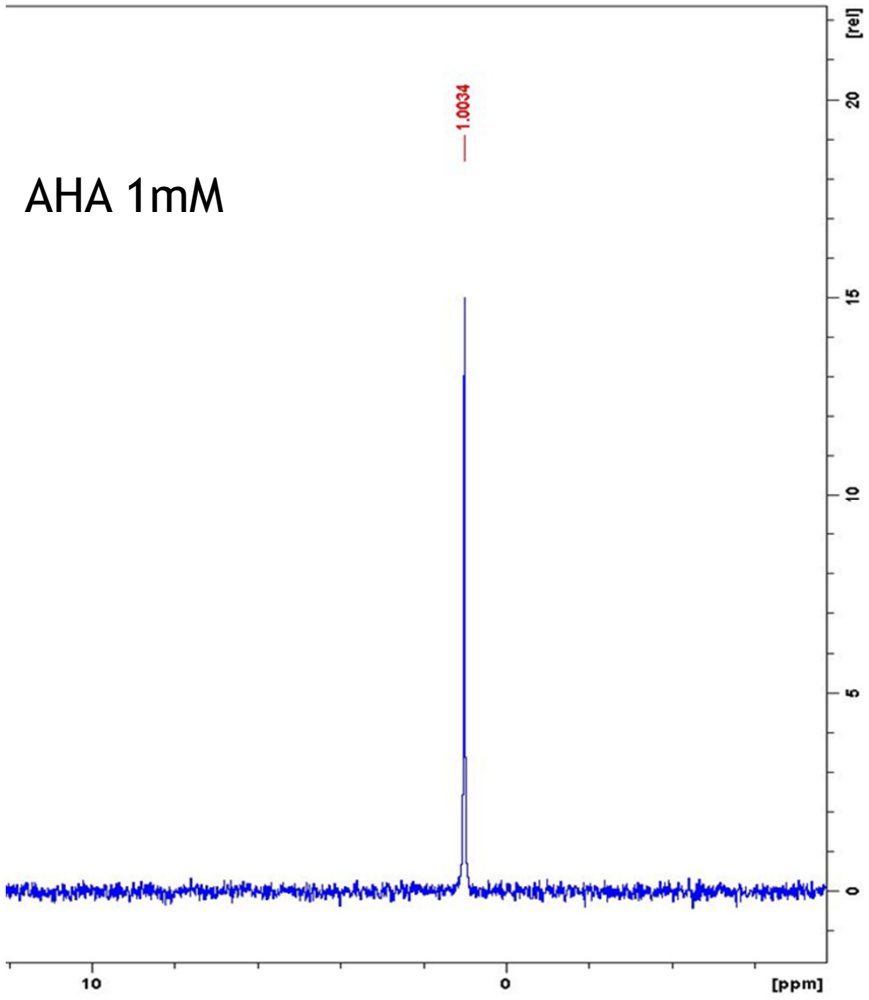
AHA

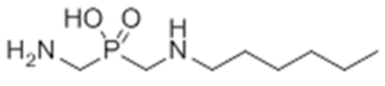




^{31}P – NMR analysis of urine sediment

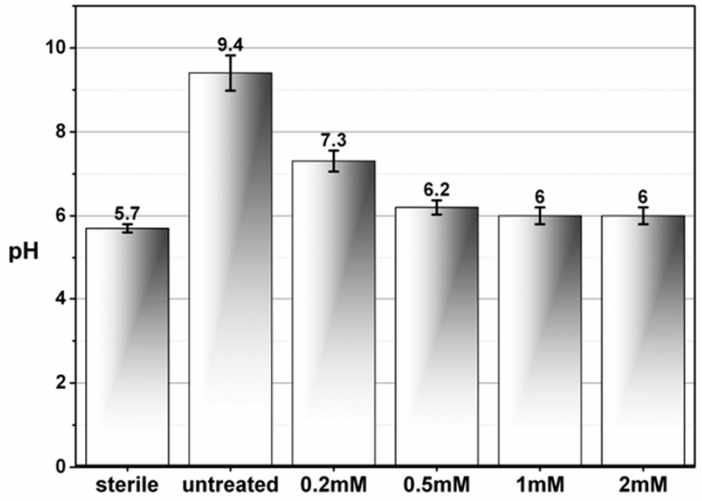
AHA 1mM

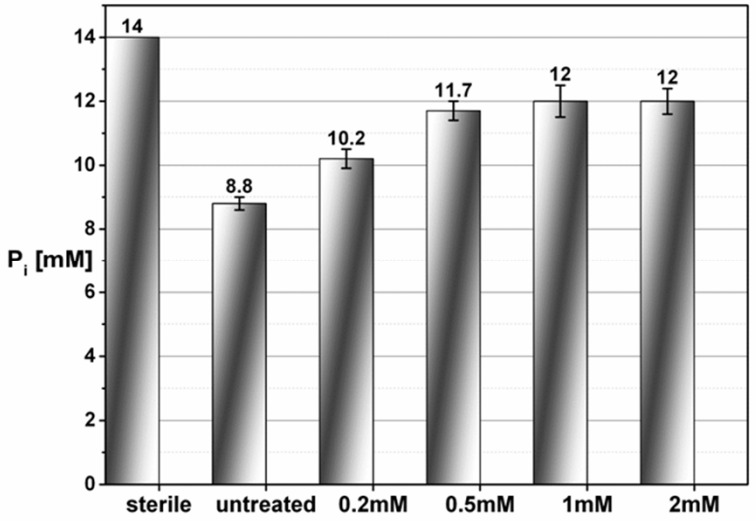




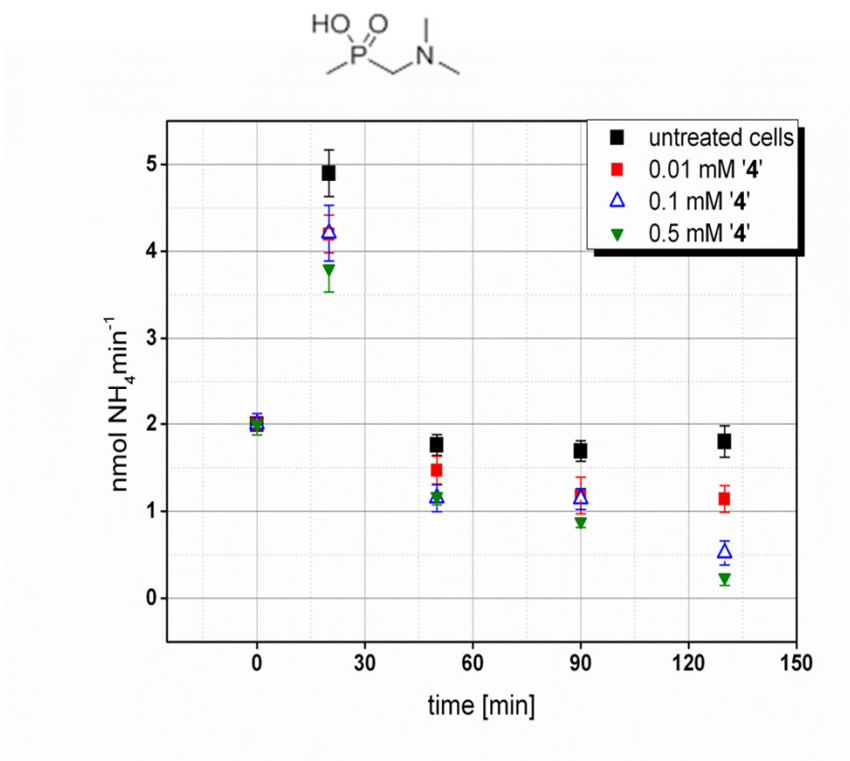
1mM

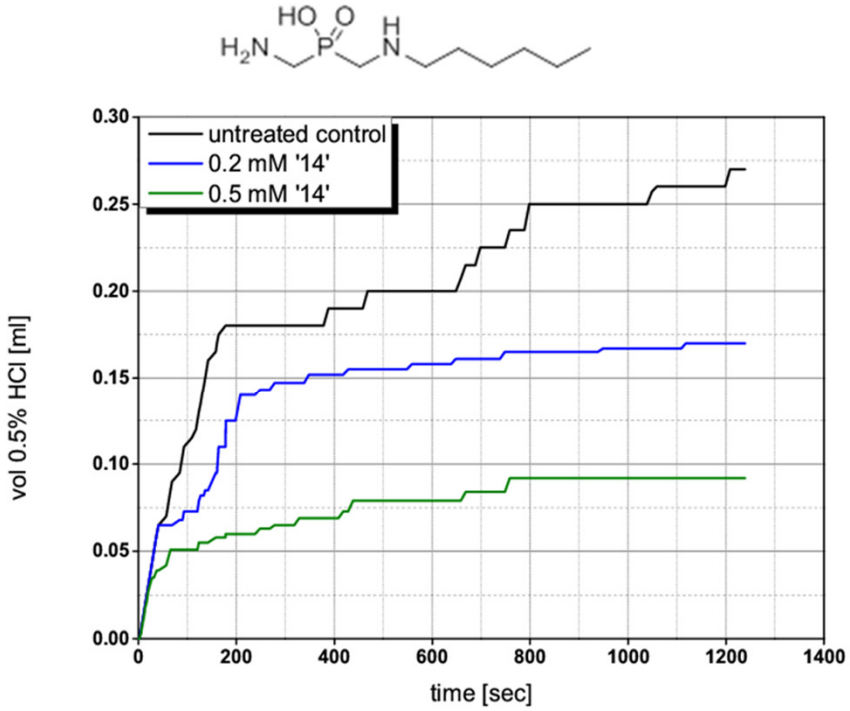
Urine stability

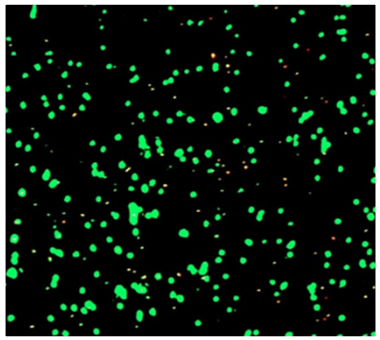




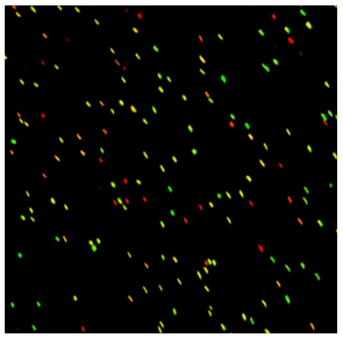
Kinetics of whole-cell urease

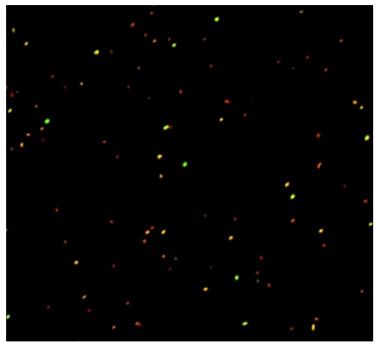


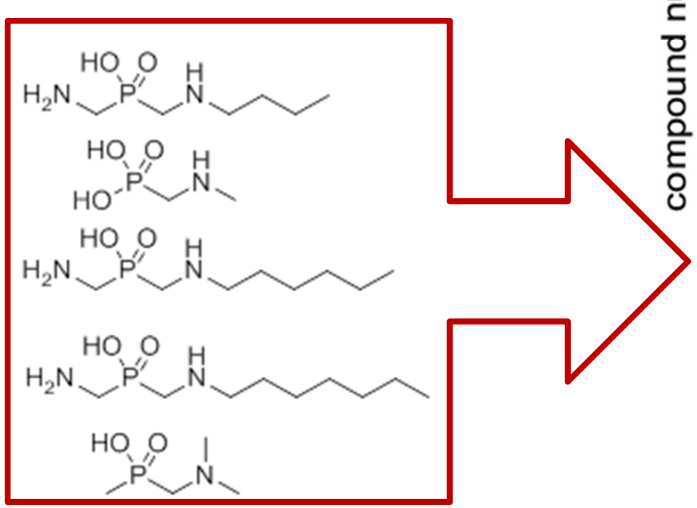


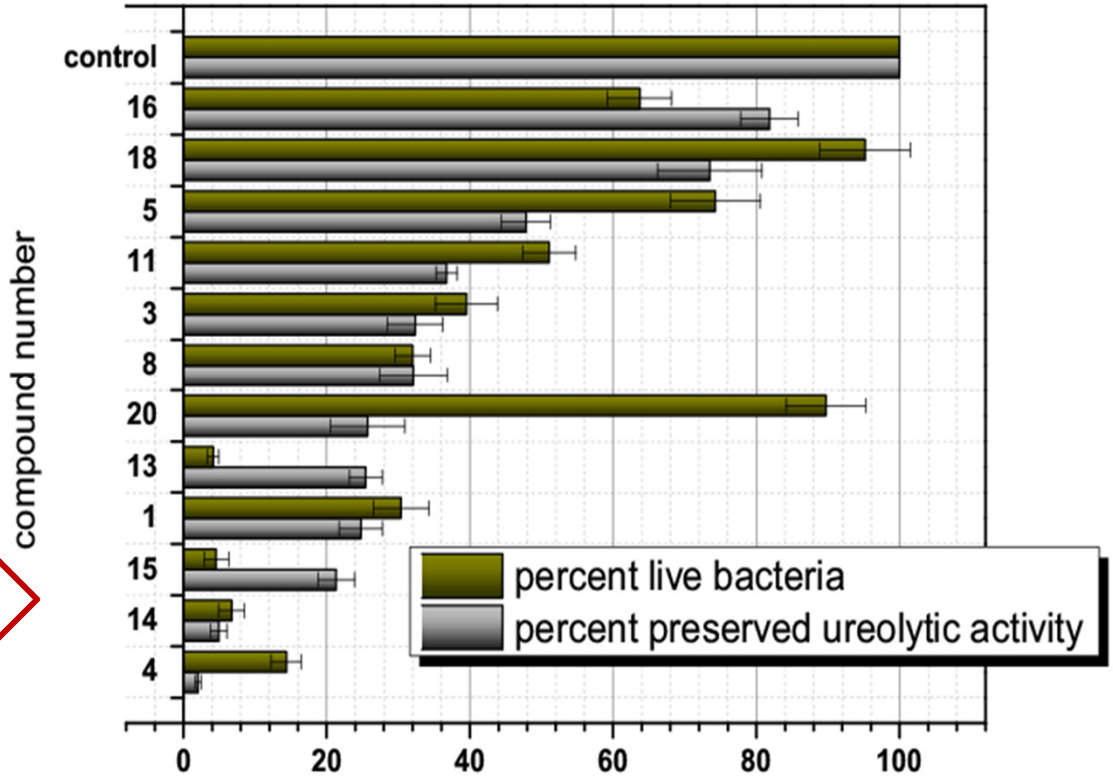


Untreated culture

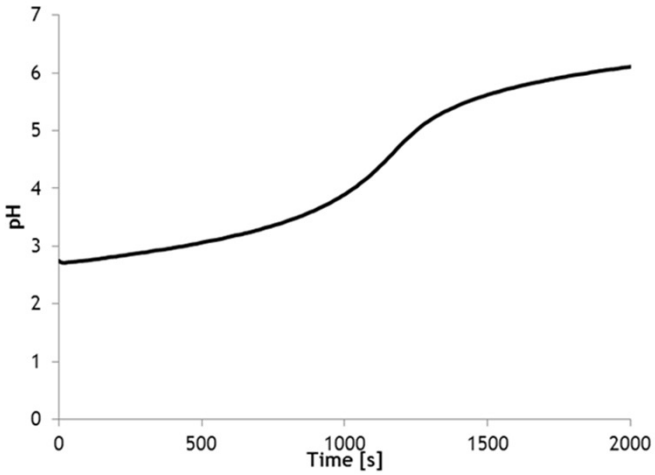


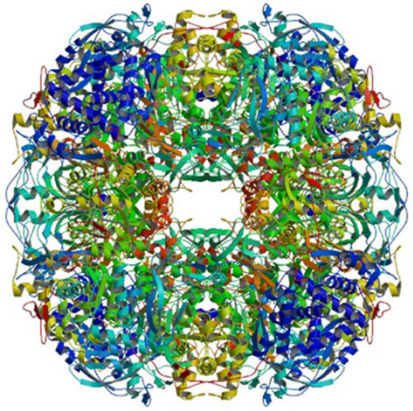






Helicobacter pylori J33





High virulence: CagA protein
VacA cytotoxin
swarming
urease

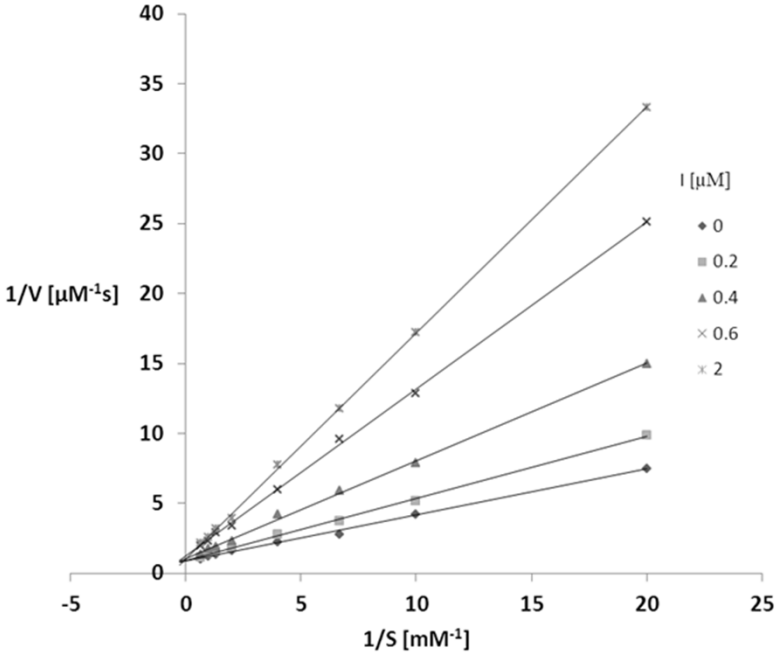
Intra- and extracellular form

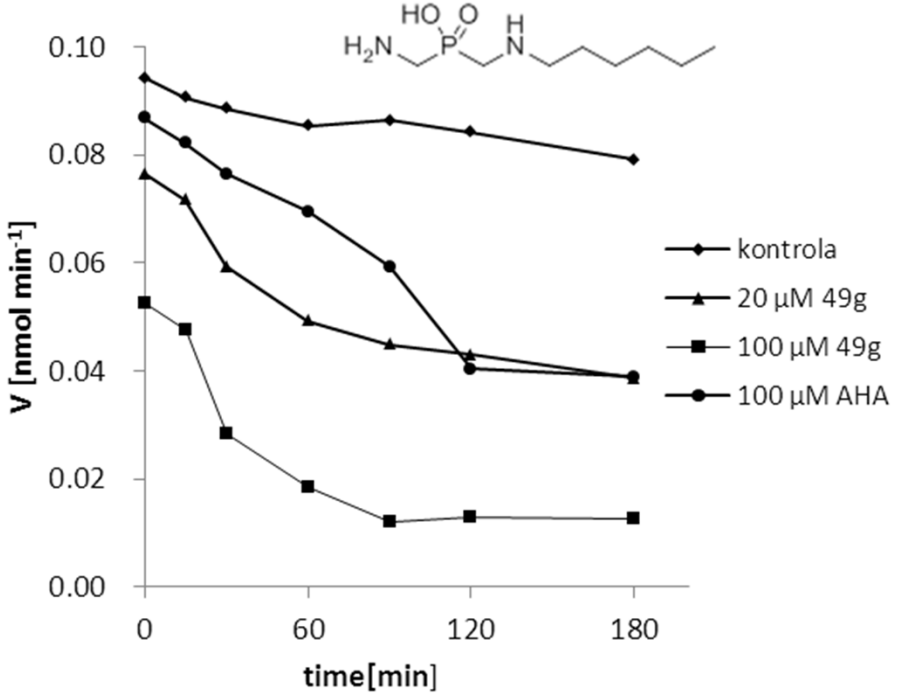
Up to 10% cell protein, constitutive, Km~0.3mM

Protection from acid
Ni scavenging

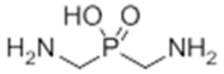
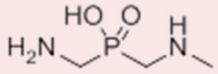
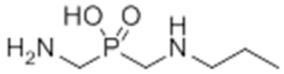
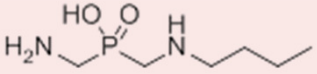
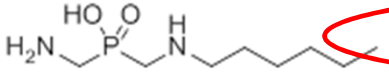
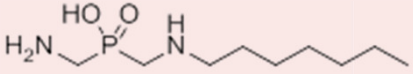
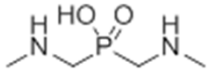
Ha CN, Oh ST, Sung JY, Cha KA, Hyung LM, Oh BH *2001.Supramolecular assembly and acid resistance of Helicobacter pylori urease*. Nat Struct Biol 8(6): 505-509

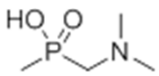
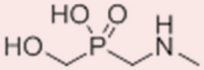
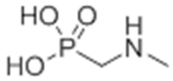
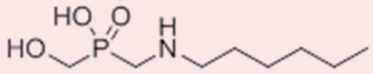
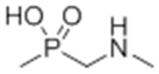
H. pylori J33 urease inhibition



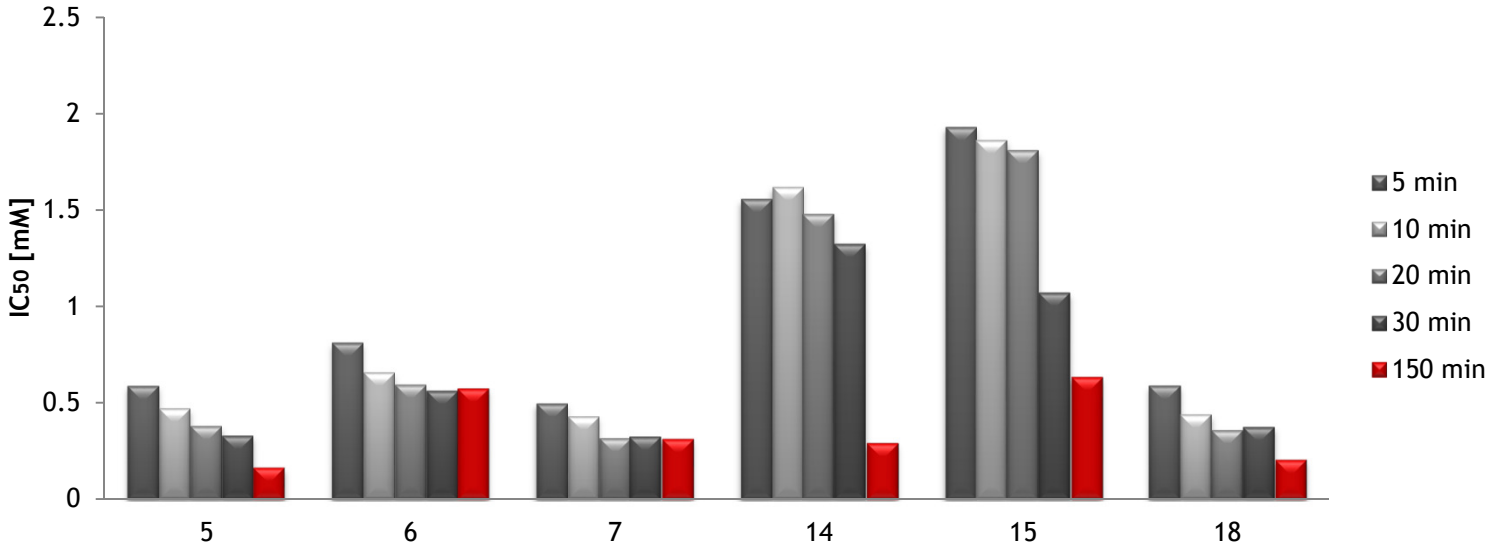


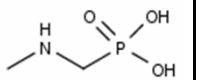
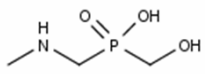
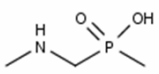
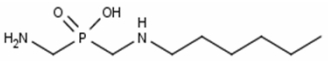
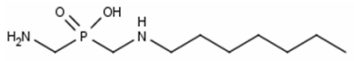
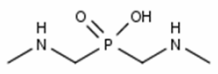
screening

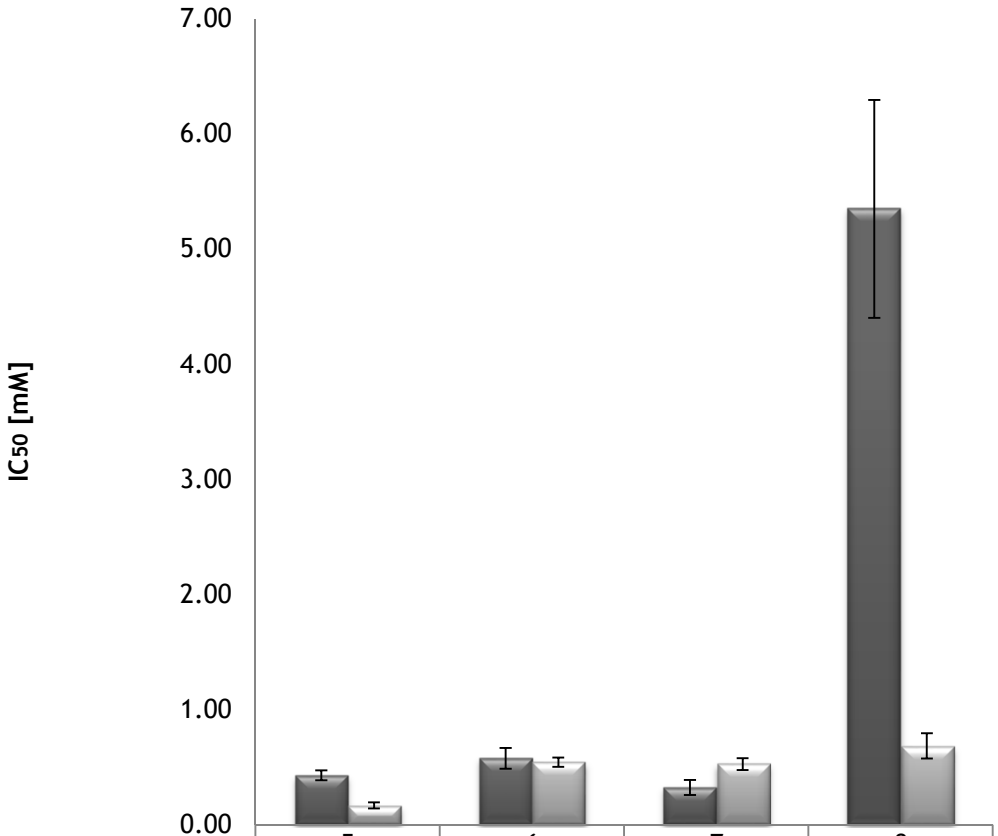
Structure	*Helicobacter pylori* (purified enzyme)		*E. coli* + pGEM::ureOP		*Helicobacter pylori* (whole cells)	
	IC50 (μM)	Ki (μM)	IC50 (μM)	IC50 2 h INC (μM)	IC 50 (μM)	IC50 2 h INC (μM)
	720,71 ± 70,11	60,98 ± 9,21	NA		<18700*	210 ± 20
	274,67 ± 20,03	20,86 ± 2,03	879.58 ± 104.83	439.71 ± 60.99	1500 ± 160	550 ± 70
	248,09 ± 20,03	22,08 ± 2,11	746.45 ±93.68	305.62 ± 43.17	<4200*	480 ± 200
	212,79 ± 15,67	27,02 ± 2,070	779.35 ± 99.26	284.09 ± 41.42	1470 ± 210	570 ± 120
	5,63 ± 0,317	0,29 ± 0,01	186.36 ±29.59	16.92 ±2.23	1500 ± 200	410 ± 200
	1506,7 ± 114,15	877,6 ± 24,7	3357.17 ± 568.21	2256.42 ± 350.99	1460 ± 400	640 ± 30
	219,23 ± 18,67	26,14 ± 1,78	1257.64 ± 187.86	269.48 ± 38.51	470 ± 80	210 ± 80

Structure	*Helicobacter pylori* (purified enzyme)		*E. coli* + pGEM::ureOP		*Helicobacter pylori* (whole cells)	
	IC50 (μM)	Ki (μM)	IC50 (μM)	IC50 2 h INC (μM)	IC 50 (μM)	IC50 2 h INC (μM)
	34.25 ± 2.95	9.27 ± 0.35	179.35 ± 32.29	25.06 ±4.86	<9570*	690 ± 150
	3.56 ± 0.321	1.03 ± 0.068	759.37 ±106.43	429.72 ± 62.74	600 ± 170	580 ± 80
	179.43 ± 12.94	38.29 ± 1.08	163.85 ±24.12	110.28 ±21.13	520 ± 170	170 ± 30
	381.03 ± 31.46	74.26 ± 4.65	-	-	250 ± 2	250 ± 100
	344.23 ± 17.61	61.64 ± 3.52	573.29 ±76.02	306.22 ±48.38	350 ± 130	510 ± 130

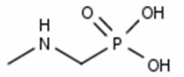
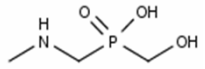
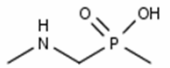
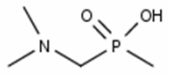
Time of incubation

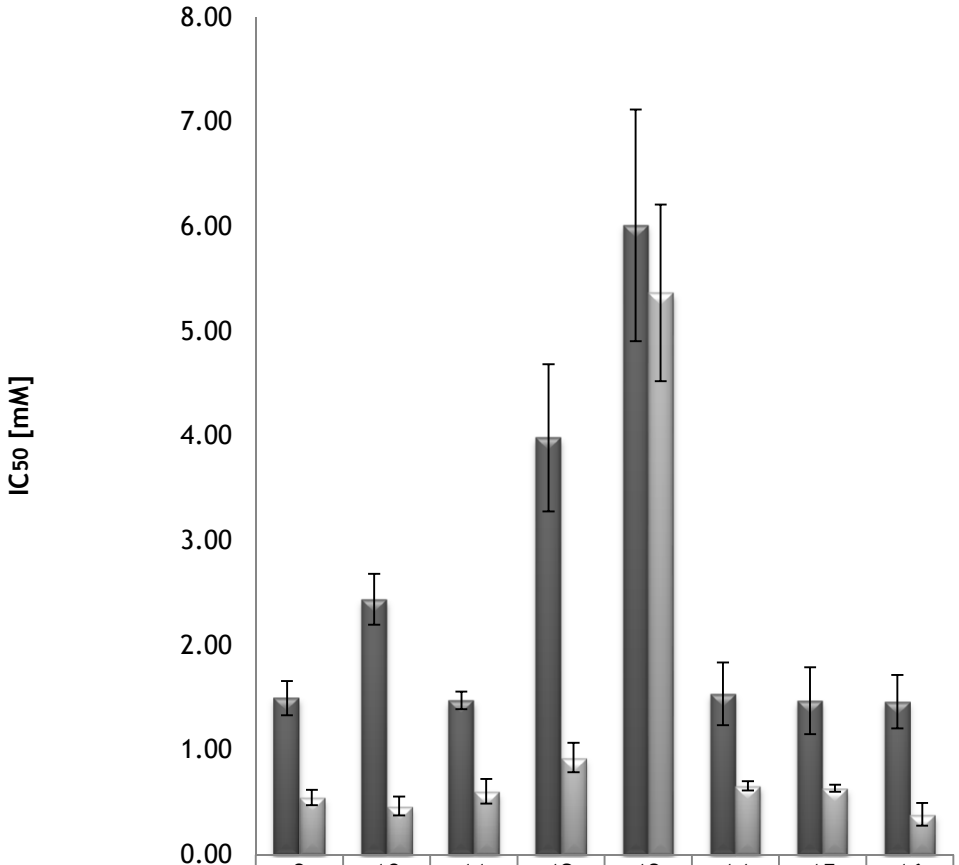


5	6	7	14	15	18
					

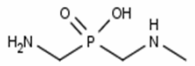
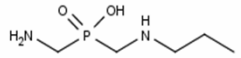
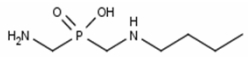
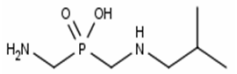
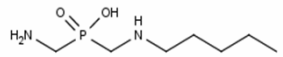
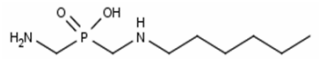
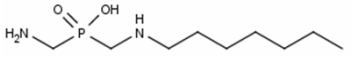
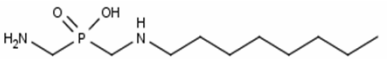


	5	6	7	8
No preincubation	0.43	0.58	0.32	5.35
2h preincubation	0.17	0.54	0.53	0.69

Nr	Inhibitor
5	
6	
7	
8	



	9	10	11	12	13	14	15	16
No preincubation	1.50	2.44	1.47	3.98	6.01	1.54	1.47	1.46
2h preincubation	0.55	0.46	0.61	0.93	5.37	0.66	0.64	0.39

Nr	Inhibitor
9	
10	
11	
12	
13	
14	
15	
16	

Summary

Aminophosphinates are efficient inhibitors of bacterial urease

They prevent struvite crystallization in artificial urine infection model

H. pylori cells are more resistant to urease inhibition

Studied structures are analogs of aminoacids and urease transition state

Aminophosphinates are hydrolytically stable and resistant to microbial degradation

Aminophosphonates possess chelating properties

Amphiphilic structures affect OM integrity

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THANK YOU !