Three novel bactericidal compounds specific against *Helicobacter pylori*

Zaragoza, 6 de junio de 2012

Content

Programa Cooperación Farma-Biotech

Three novel bactericidal compounds specific against *Helicobacter pylori*




Javier Sancho
Adrián Velázquez-Campoy
José Alberto Carrodeguas
Ramón Hurtado

protein targets

GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA E INNOVACIÓN

GOBIERNO DE ARAGON

CTP

CSIC

i+cs Instituto Aragonés de Ciencias de la Salud

searteriosclerosis.org SOCIEDAD ESPAÑOLA DE ARTERIOSCLEROSIS

Programa Cooperación Farma-Biotech

Three novel bactericidal compounds specific against *Helicobacter pylori*




Javier Sancho
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2009

protein targets

GOBIERNO DE ARAGON

- Protein Folding and Molecular Design
- Biomolecular Interactions
- Stem cells and apoptosis
- Glycosyltransferases and hydrolases in human diseases

Programa Cooperación Farma-Biotech

Three novel bactericidal compounds specific against *Helicobacter pylori*

2009

protein targets

•**Protein Folding and Molecular Design**

1996

Programa Cooperación Farma-Biotech

Three novel bactericidal compounds specific against *Helicobacter pylori*

2009

protein targets

CIENCIAS ZARAGOZA

bifi

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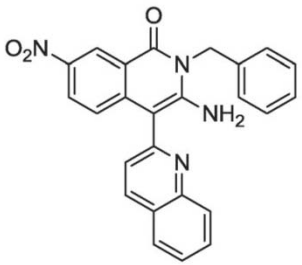
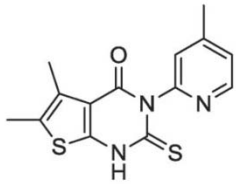
Three novel bactericidal compounds specific against *Helicobacter pylori*

From cloning to structure and simulation

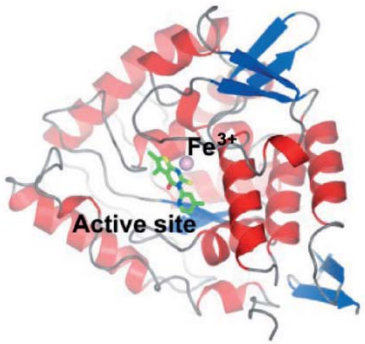


compound III



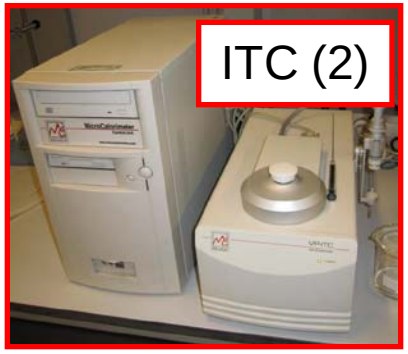
compound IV

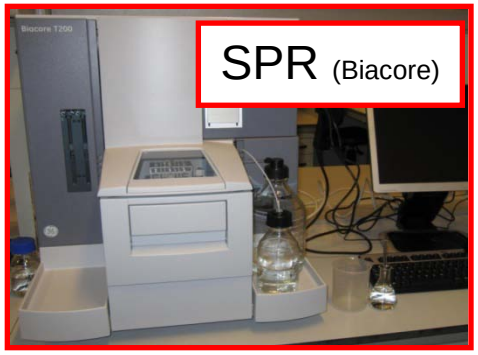
C)



Protein stability and ligand binding

Binding

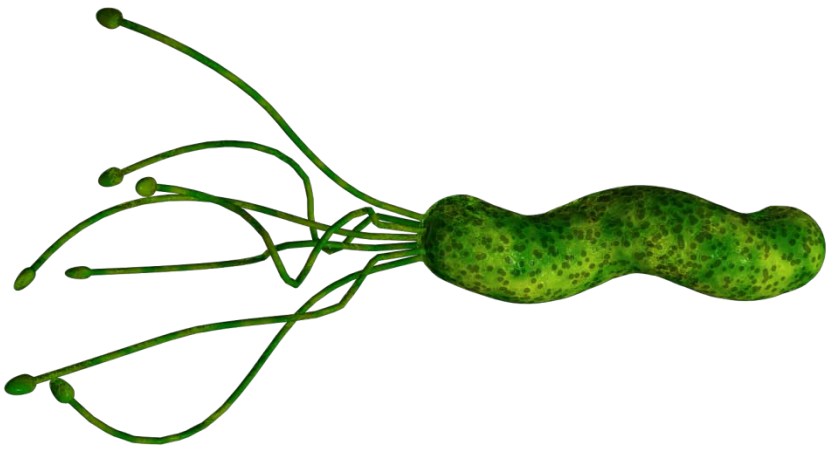




Stability

2. The Product

a) Target indications



***H pylori* is infecting 50% of global population**

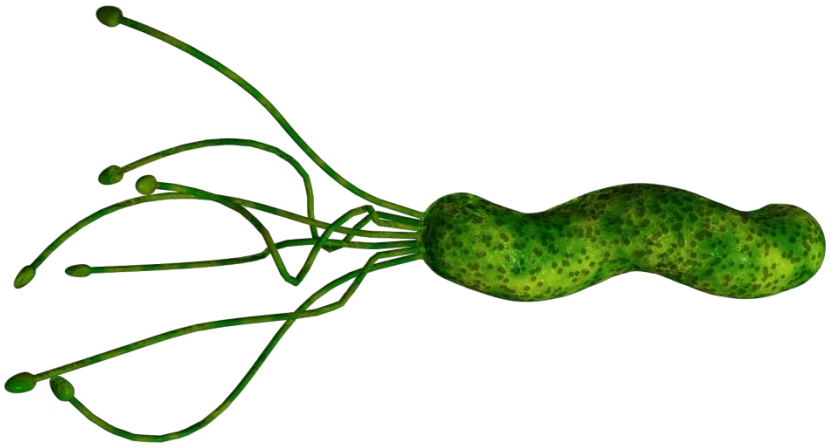
- 2010 worldwide prevalence between 7% and 87%.
- Average prevalence in Europe around 30%.

Individuals infected with *H. pylori* have a

- 10 to 20% lifetime risk of developing peptic ulcers
- 1 to 2% risk of acquiring stomach cancer

2. The Product

a) Target indications



Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report
Malfertheiner et al. *Gut* **56**:772–781 (2007)

Eradication of *H pylori* infection is recommended in:

(a) peptic ulcer disease

(b) low grade gastric, mucosa associated lymphoid tissue (MALT) lymphoma

(c) atrophic gastritis

(d) first degree relatives of patients with gastric cancer

(e) unexplained iron deficiency anaemia

(a) chronic idiopathic thrombocytopenic purpura

Eradication of *H pylori* infection **may prevent** peptic ulcer in naïve users of non-steroidal anti-inflammatory drugs (NSAIDs).

2. The Product

a) Target indications

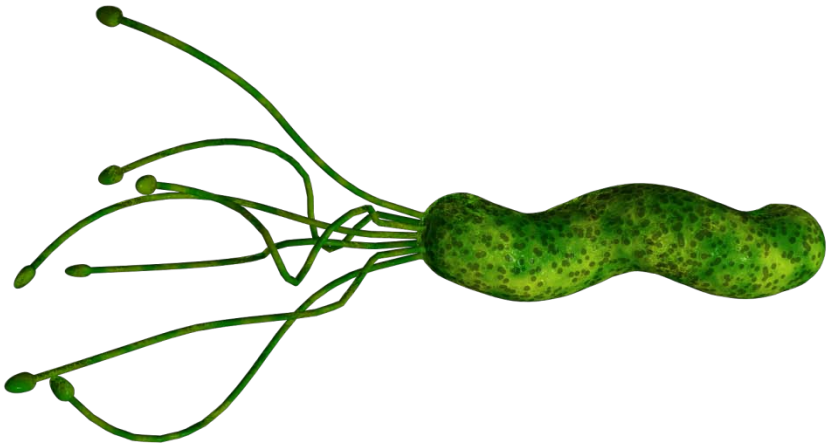
Management of Helicobacter pylori infection-the Maastricht IV/ Florence Consensus Report
Malfertheiner et al. *Gut* **61**:646-664 (2012)

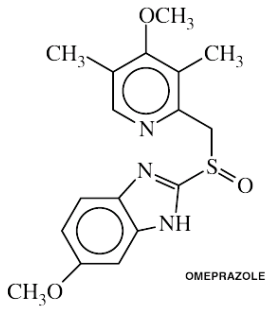
The triple treatment including PPI-clarithromycin and amoxicillin or metronidazole **… has become universal.**

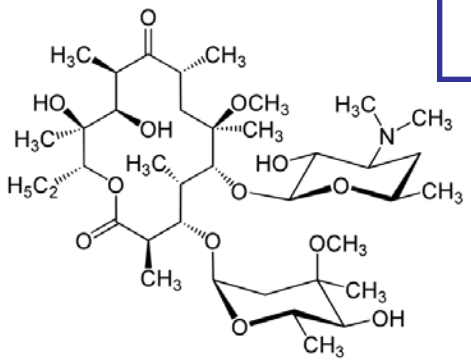
However, the most recent data show **that this combination has lost some efficacy** and often allows the cure of only a maximum of **70%** of the patients.

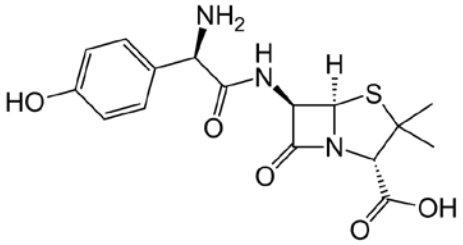
No new drug has been developed for this indication.

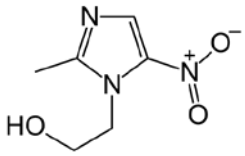
There is not a single specific *H. pylori* antimicrobial





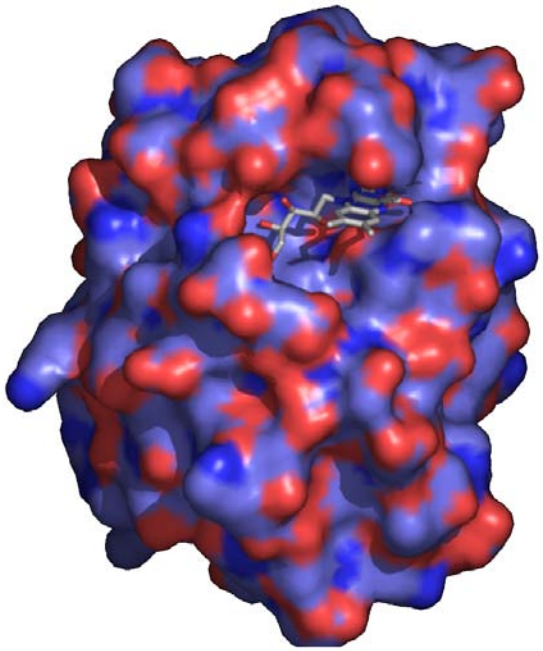






2. The Product

b) Innovative mechanisms of action



Crystal structure of oxidized **flavodoxin, an essential protein in *Helicobacter pylori***
Freigang et al.
Protein Science (2002), 11:253-261.

Flavodoxins are bacterial proteins involved in different redox reactions

PS I → Fld → FNR → $NADP^+$

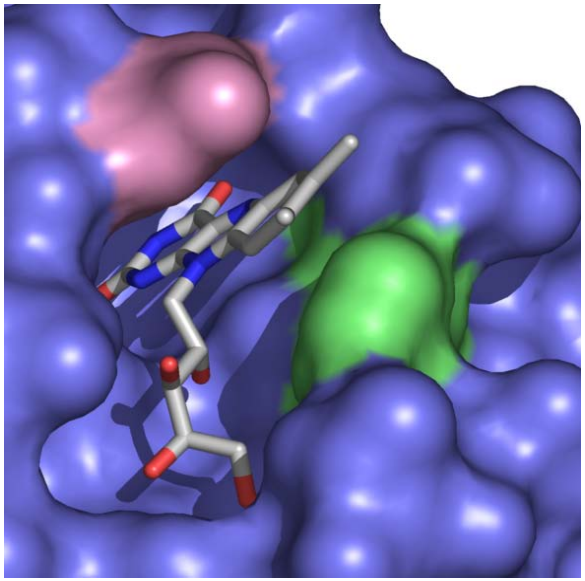
Fld → Nitrogenase → N_2

Fld — Activation of enzymes → Methionine synthase; Ribonucleotide reductase; Piruvate-formiate lyase; HMBPP synthase

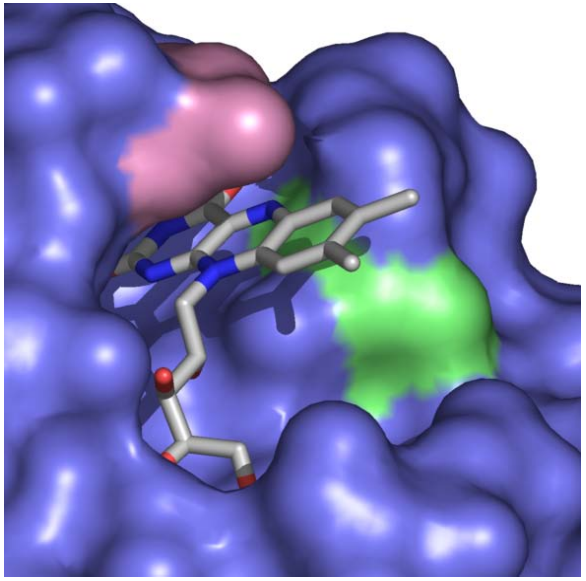
Fld → Flavodoxin reductase → Biotine synthase → dethiobiotine

Flavodoxins: sequence, folding, binding, function and beyond
Sancho J.
Cell Mol Life Sci. (2006) 63:855-64.

Other flavodoxins



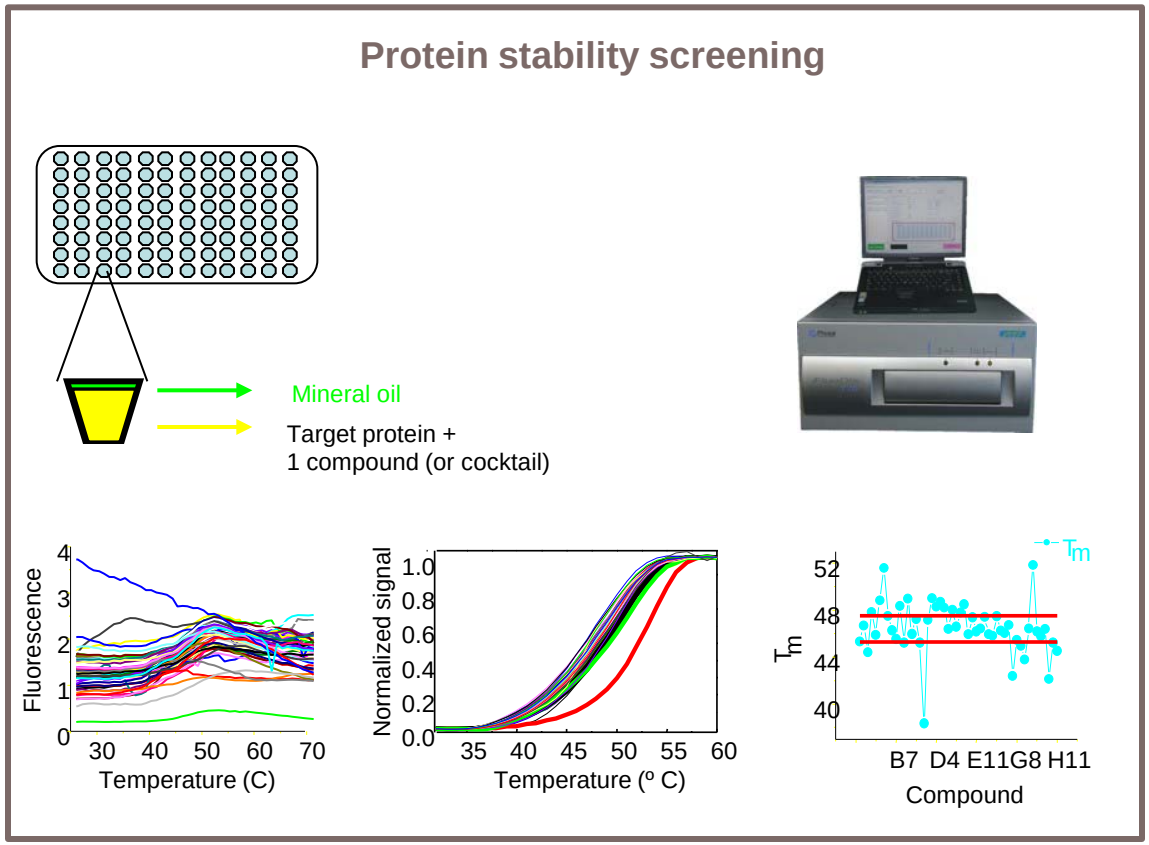
H. pylori flavodoxin



H. pylori flavodoxin has an unusual pocket at the active site

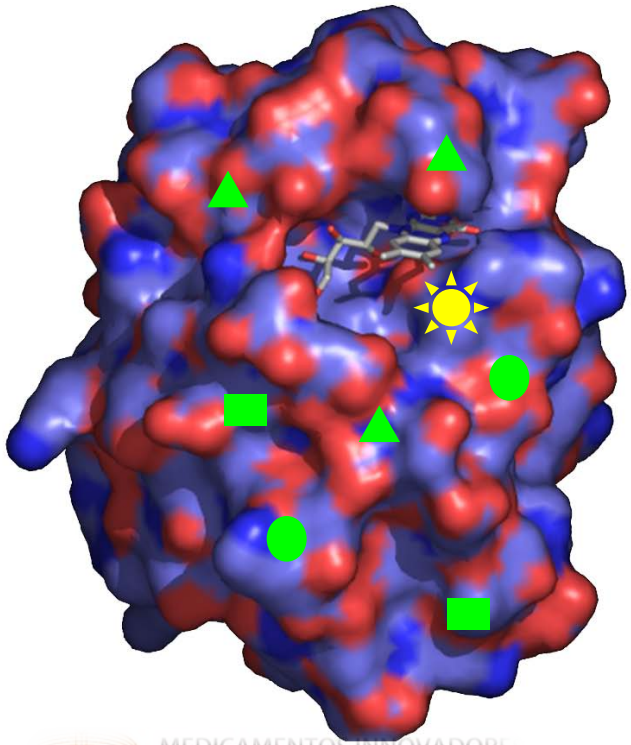
A two-step screening of commercial libraries for inhibitors

Step 1: Finding binders using a HTP protein stabilization assay



A two-step screening of commercial libraries for inhibitors

Step 1: Finding binders using a HTP protein stabilization assay



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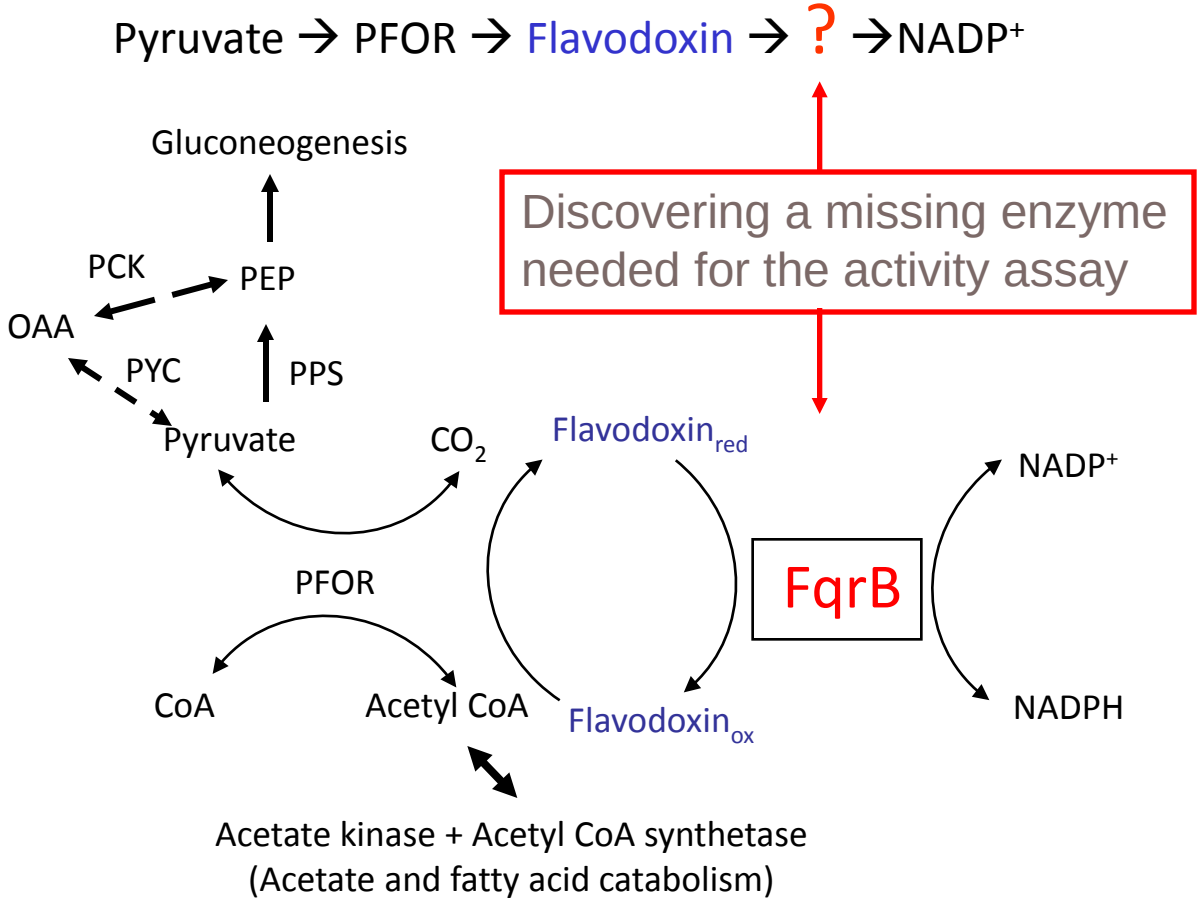
Three novel bactericidal compounds specific against *Helicobacter pylori*

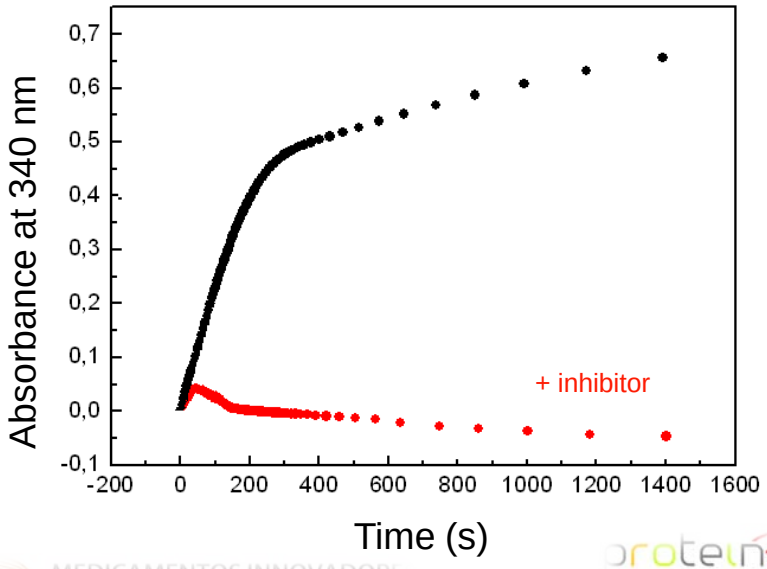


Flavodoxin: Quinone Reductase (FqrB): A Redox Partner of Pyruvate: Ferredoxin Oxidoreductase that Reversibly Couples Pyruvate Oxidation to NADPH Production in *Helicobacter pylori* and *Campylobacter jejuni*
M. St. Maurice, N. Cremades, M. A. Croxen, G. Sisson, J. Sancho and P. S. Hoffman.
J. Bacteriol. 189:4764-4773 (2007).

A two-step screening of commercial libraries for inhibitors

Step 1: Finding binders using a HTP protein stabilization assay
Step 2: Specific inhibitory assay

Pyruvate → PFOR → Flavodoxin → FqrB → $NADP^+$



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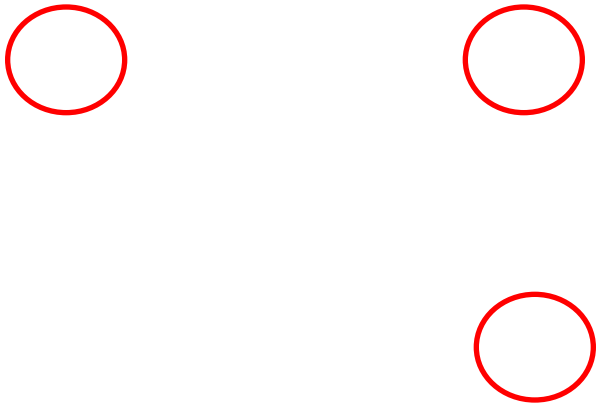
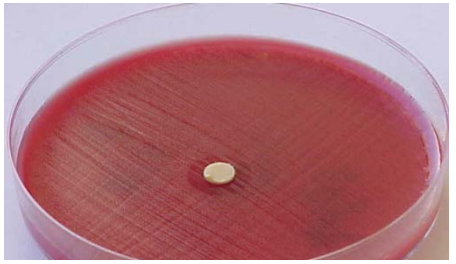
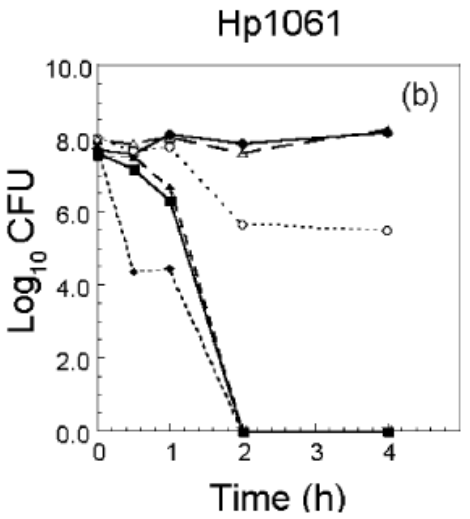
Three novel bactericidal compounds specific against *Helicobacter pylori*

Four *In vitro* flavodoxin inhibitors



The four inhibit H. pylori growth





Three are bactericidal

Cremades et al. 2009
Discovery of Specific Flavodoxin Inhibitors as Potential Therapeutic Agents against *Helicobacter pylori* Infection
ACS Chem. Biol. 4: 928-938 (2009)

Innovative Mechanism of action: Specific inhibitors of flavodoxin that kill *H. pylori*

2. The Product

c) Differential features facing the market

TABLE 2. Minimal Inhibitory Concentrations[a]

		Species							
		H. pylori 1061		*H. pylori 26695*		*C. jejuni*		*Escherichia coli* DH5α	
Inhibitor	MW	μg mL^{-1}	μM	μg mL^{-1}	μM	μg mL^{-1}	μM	μg mL^{-1}	μM
I	338	0.25	0.7	0.125	0.4	2	6	>32	>95
II	285	0.5	1.8	0.25	0.9	2	7	>32	>112
III	297	8	27	16	54	8	27	>32	>108
IV	317	2	6	1	3	1	3	>32	>101
NTZ[b]	307	4	13	1	3	8	26		
Amp.[b]	349	<0.025	<0.07	<0.025	<0.07	0.092	0.3		
DMSO		-		-		-			

[a]Minimal inhibitory concentration represents the drug concentration which inhibits growth of the bacteria.
[b]NTZ, nitazoxanide; Amp., ampicillin.

Differential features: First antimicrobials specific against *Helicobacter pylori*
No resistance yet developped
Simple chemicals

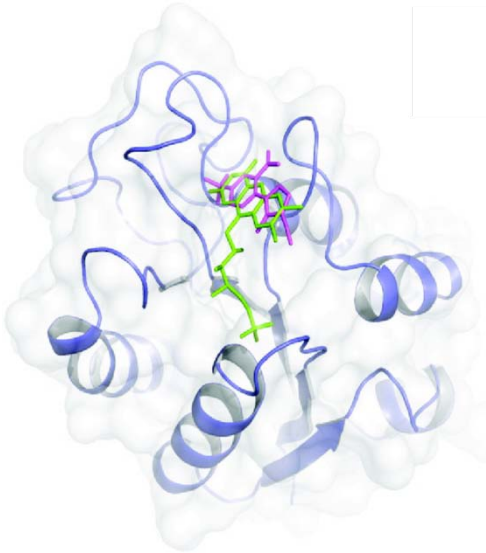
2. The Product

d) Current status of development

TABLE 1. Affinities of flavodoxin/inhibitor complexes

	Thermal upshift assay			ITC	
Inhibitor	ΔT_m[a]	K_D[b] μM	ΔG_b kcal mol^{-1}	K_D[c] μM	ΔG_b kcal mol^{-1}
I	7	12.2	−6.7	1.6 ± 0.3	−7.9 ± 0.1
II	3	65.2	−5.7	8 ± 2	−6.9 ± 0.2
III	8	8.1	−7.0	0.6 ± 1	−8.5 ± 0.7
IV	11	3.5	−7.4	3 ± 1	−7.5 ± 0.1

[a] T_m values calculated as described in the high-throughput screening section of the Methods. [b] K_D values extrapolated at 25.0 °C. [c] K_D values determined at 25.0 °C



2. The Product

d) Current status of development

HeLa cells

Inhibitor	MIC (µM)	MCC (µM)	Therap. Index (MCC/MIC)
I	1.2	6	5
II	1.2	3.5	3
IV	9.5	1.7	0.2

2. The Product

d) Current status of development

HeLa cells

Inhibitor	MIC (µM)	MCC (µM)	Therap. Index (MCC/MIC)
I	1.2	6	5
II	1.2→0.27	3.5→10	3→38
IV	9.5→8.5	1.7→100	0.2→12

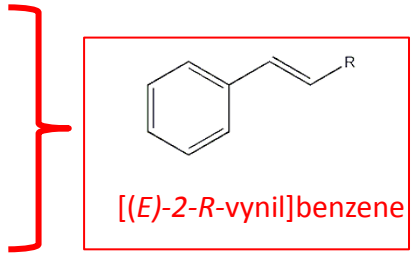
Variants designed and tested with increased Therapeutic Index

Compound	Kd (µM)	Toxicity H. pylori (MIC µM)	Citotoxicity HeLa MCC (µM)	Therapeutic Index (MCC/MIC)
1	3.5	2.4	3.5	1.5
2	8	40	40	1.0
3	5.5	2.40	0.70	0.3
4	10	8	0.01	0.001
5	8.8	2.4	0.1	0.04
6	0.67	2.4	2	0.8
7	3.73	150	100	0.7
8	16	4.8	1	0.2
9	19	4.8	10	2.0
10	10.98	75	100	1.3
11	2.78	1.06	5	4.7
12	4.44	0.53	8	15.1
13	7.38	0.53	20	**37.8**
14	4.14	0.53	8	15.1
15	6.61	1.06	10	9.4
16	40	1.06	20	18.9
17	1	1.2	6	5.0
18	0.43	2.4	1	0.4
19	1.5	2.4	6	2.5
20	1.66	2.4	6	2.5
21	0.78	1.2	10	8.3
22	1.92	7.5	70	9.3
23	25	2	10	5.0
24	1.84	150	12	0.08

2. The Product

d) Current status of development

QSAR models for:
- affinity (*LogKd*)
- inhibitory activity (*Log(1/MIC)*



Development of QSAR models for Fld-binding and inhibitory features of [(E)-2-R-vinyl]benzene derivatives in *Helicobacter pylori* associated diseases.
JJ Galano &J. Sancho. In Prep.

2. The Product

d) Current status of development

A. Lanas
U. Zaragoza/I+CS

No sign of toxicity in mice
(1-10 mg/kg, twice a day for 5 days)

2. The Product

d) Current status of development

Jerboa as models to test efficiency for erradication
(70 % infectivity rates)

2. The Product

e) IPR protection

NÚMERO DE SOLICITUD	P200700566	
TÍTULO	Uso de compuestos como inhibidores de la Flavodoxina de Helicobacter	
INVENTORES:	Javier Sancho, Nunilo Cremades, Adrián Velázquez	
FECHA DE SOLICITUD	23/02/2007	
EXTENSIONES /FECHA DE SOLICITUD	PCT/ES/2008/000093 Fecha de solicitud:20/02/2008	
	EP08736697.7	27/08/2009
	JP2009-550299	21/08/2009
	US12/528,278	21/08/2009
SITUACIÓN ACTUAL	5ª anualidad en Europa:	Pagada
	Japón:	Realizada la Solicitud de examen
	USA:	Patente concedida 20/12/2011

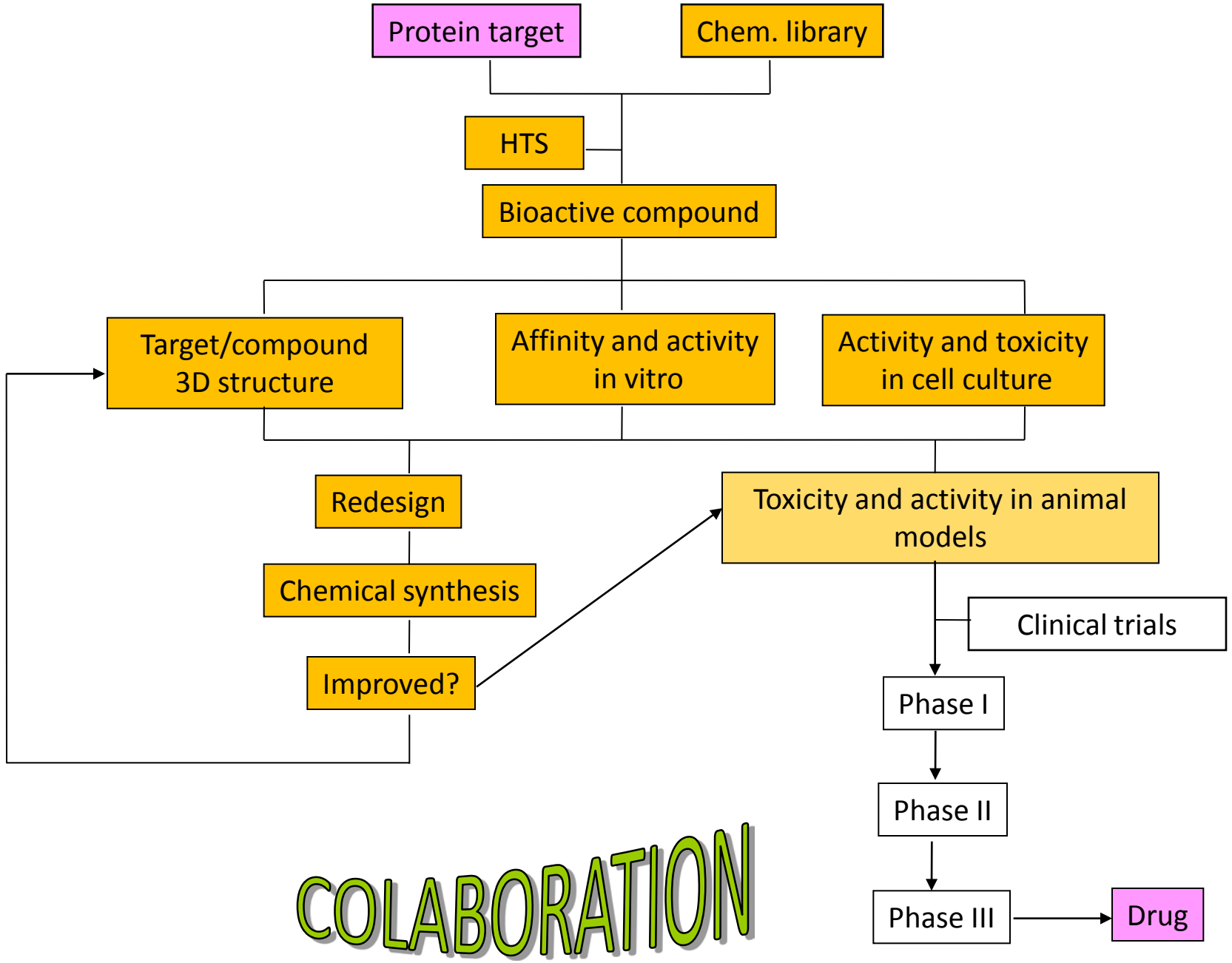
NÚMERO DE SOLICITUD	P200700645	
TÍTULO	Composición para el tratamiento de enfermedades infecciosas	
INVENTORES:	Javier Sancho, Nunilo Cremades, Adrián Velázquez	
FECHA DE SOLICITUD	02/03/2007	
EXTENSIONES /FECHA DE SOLICITUD	PCT/ES2008/000098	22/02/2008
	EP08750368.6	27/08/2009
	JP2009-551231	27/08/2009
	US12/529,626	02/09/2009
SITUACIÓN ACTUAL	5ª anualidad en Europa:	Pagada
	Japón:	Realizada la Solicitud de examen
	USA:	Pendiente de concesión

NÚMERO DE SOLICITUD	P200700646	
TÍTULO	Composición para el tratamiento de enfermedades infecciosas causadas por Helicobacter	
INVENTORES:	Javier Sancho, Nunilo Cremades, Adrián Velázquez	
FECHA DE SOLICITUD	02/03/2007	
EXTENSIONES /FECHA DE SOLICITUD	PCT/ES2008/000097	22/02/2008
	EP08750367.8	29/09/2009
	JP2009-551230	28/09/2009
	US12/529,679	02/09/2009
SITUACIÓN ACTUAL	5ª anualidad en Europa:	Pagada
	Japón:	Realizada la Solicitud de examen
	USA:	Pendiente de concesión

2. The Product

f) Pitfalls & Risks to be considered

- Efficacy for erradication in animal models and humans remains to be tested
- Toxicity in humans remains to be tested


Protein target
Chem. library
HTS
Bioactive compound
Target/compound 3D structure
Affinity and activity in vitro
Activity and toxicity in cell culture
Redesign
Chemical synthesis
Improved?
Toxicity and activity in animal models
Clinical trials
Phase I
Phase II
Phase III
Drug
COLABORATION

3. Partnering Opportunities

JAVIER SANCHO: jsancho@unizar.es

OTRI-UNIZAR: Eduardo Almenara. almenara@unizar.es
Elena Tobías. etobias@unizar.es

The University of Zaragoza seeks to have these compounds marketed

We contemplate to out-license the technology to a strategic partner in order to:
Complete pre-clinical assays
Start clinical trials in humans

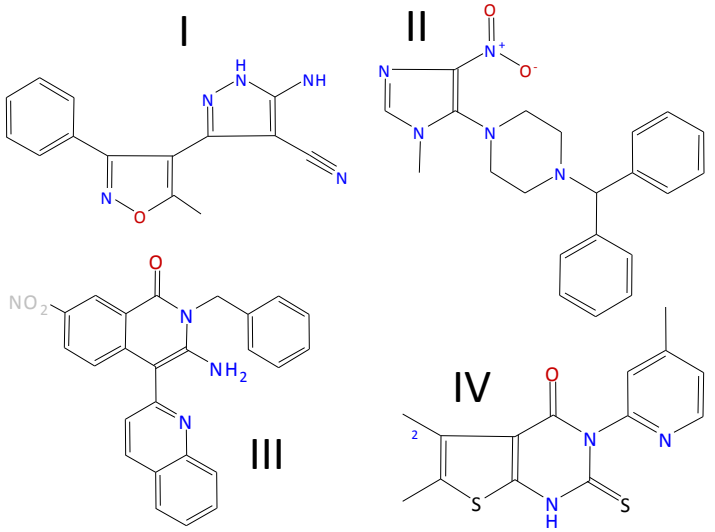
We are open to analyze different forms of collaborations with your company

4. Partnering Opportunities for other proprietary small molecules

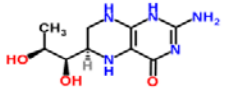
Phenylketonuria (PKU)

Patented pharmacological chaperones (alternatives to KUVAN)

INVENTORES (p.o. de firma): A.Pey, Mi.Ying, N.Cremades, A.Velázquez, J.Sancho, A.Martínez
TITULO: Compositions for the treatment of hyperphenylalaninemia.
Nº DE SOLICITUD: 070126826-1216
País de prioridad: Noruega
Fecha de prioridad: 28/06/2007
Países a los que se ha extendido: UE, USA, Canadá, Japón
ENTIDAD TITULAR: Universidad de Zaragoza



BIOMARIN™



Kuvan

KUVAN® is approved to reduce blood Phe levels in patients with hyperphenylalaninemia (HPA) due to tetrahydrobiopterin- (BH4-) responsive Phenylketonuria (PKU).

KUVAN is to be used in conjunction with a Phe-restricted diet.

4. Partnering Opportunities for other proprietary small molecules

Alzheimer

Patented inhibitors of Aβ1-42 aggregation (effective in two fungi models)

ENTIDAD TITULAR: Universidad de Zaragoza (80 %) , Universidad Autónoma de Barcelona (20 %)
INVENTORES Javier Sancho, Laura C. López, José Alberto Carrodeguas, Salvador Ventura
TITULO: Compuestos inhibidores de la agregación del peptido beta amiloide
Nº DE SOLICITUD: P201132020
País de prioridad: España
Fecha de prioridad: 15/12/2011

ENTIDAD TITULAR: Universidad de Zaragoza (80 %) , Universidad Autónoma de Barcelona (20 %)
INVENTORES Javier Sancho, Laura C. López, José Alberto Carrodeguas, Salvador Ventura
TITULO: Agente inhibidor de la agregacion del peptido beta amiloide
Nº DE SOLICITUD: P201131955
País de prioridad: España
Fecha de prioridad: 02/12/2011

Steem cell technology

Patented compounds inducing selective death of pluripotent stem cells

ENTIDAD TITULAR: Universidad de Zaragoza, University of Cologne, Instituto Aragonés de Ciencias de la Salud.
INVENTORES (p.o. de firma): José Alberto Carrodeguas, Javier Sancho, Celia Conesa, Agapios Sachinidis
TITULO: Uso de derivados de bencetonio para la eliminación de células madre
Nº DE SOLICITUD: P201130803
País de prioridad: España
Fecha de prioridad: 18/05/2011

Three novel bactericidal compounds specific against *Helicobacter pylori*

In 2007, the market for therapetics for gastric and duodenal ulcers generated 1.500 M$

protein targets

Novel antibiotics (no resistences yet)

Specific antibiotics for Helicobacter pylori

Two different scaffolds

Zaragoza, 6 de junio de 2012