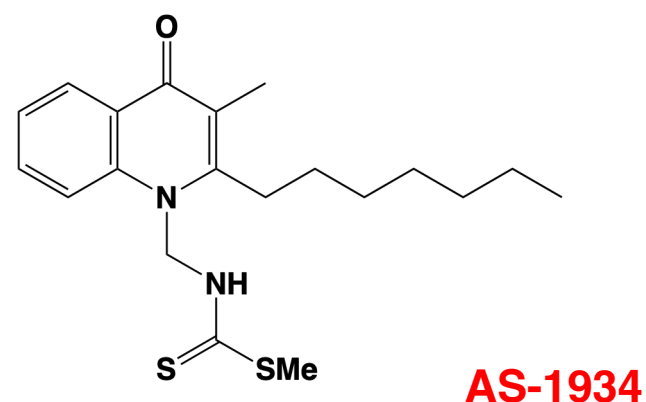
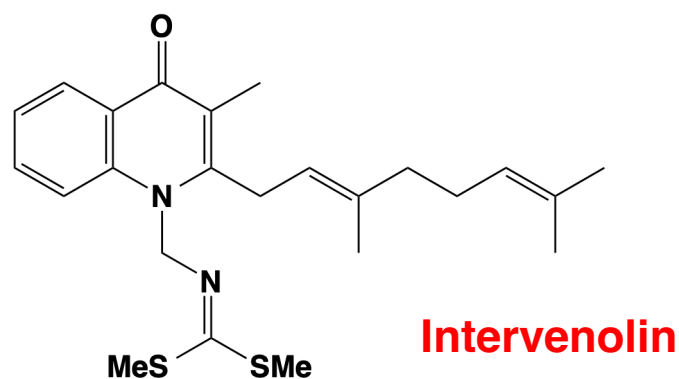


AS-1934, a Derivative of Intervenolin, Exerts Potent *H. pylori* Activity



Helicobacter pylori (*H. pylori*)

- a gram-negative, microaerophilic bacterium.
- 50 % of the world's populations are infected.
- known as a primary cause of gastric cancer.



Papagiannakis P et al. *Eur. J. Intern Med.*, 2013.

IARC, World Health Organization, 1994.

Fox A et al., *Microbiol. and Immun.*, 2014.

Table 1. Studies of non-cardia gastric cancer comparing *H. pylori* assessment by ELISA and by immunoblot

Study	Country	N cases	Follow-up (yrs)	ELISA			Immunoblot		
				% Cases positive	OR	95% CI	% Cases positive	OR	95% CI
Prospective									
Simán <i>et al.</i> , 1997 ⁵	Sweden ¹	27	5.7	89%	11.1	2.4-71.8		–	–
Simán <i>et al.</i> , 2007 ⁶	Sweden ¹	67	9.2	–	–	–	97%	17.8	4.2-74.8
Mitchell <i>et al.</i> , 2008 ⁷	Australia	34	11.6	79%	2.3	0.9-5.8	94%	10.6	2.4-47.4
González <i>et al.</i> , 2012 ⁸	Europe ²	88	10.7	82%	6.81	3.0-15.1	93%	21.4	7.1-64.4
Case-control									
Peleteiro <i>et al.</i> , 2010 ⁹	Portugal	272	–	84%	0.66	0.37-1.17	76%	0.73	0.46-1.17

¹The same cohort with different follow-up.

²Multicentric study, part of the European Prospective Investigation into Cancer and Nutrition (EPIC).

Abbreviations: OR = odds ratio; CI = confidence interval.

Plummer M et al., *Int. J Cancer*, 487-490, 2015.

Worldwide prevalence of *H. pylori* infection

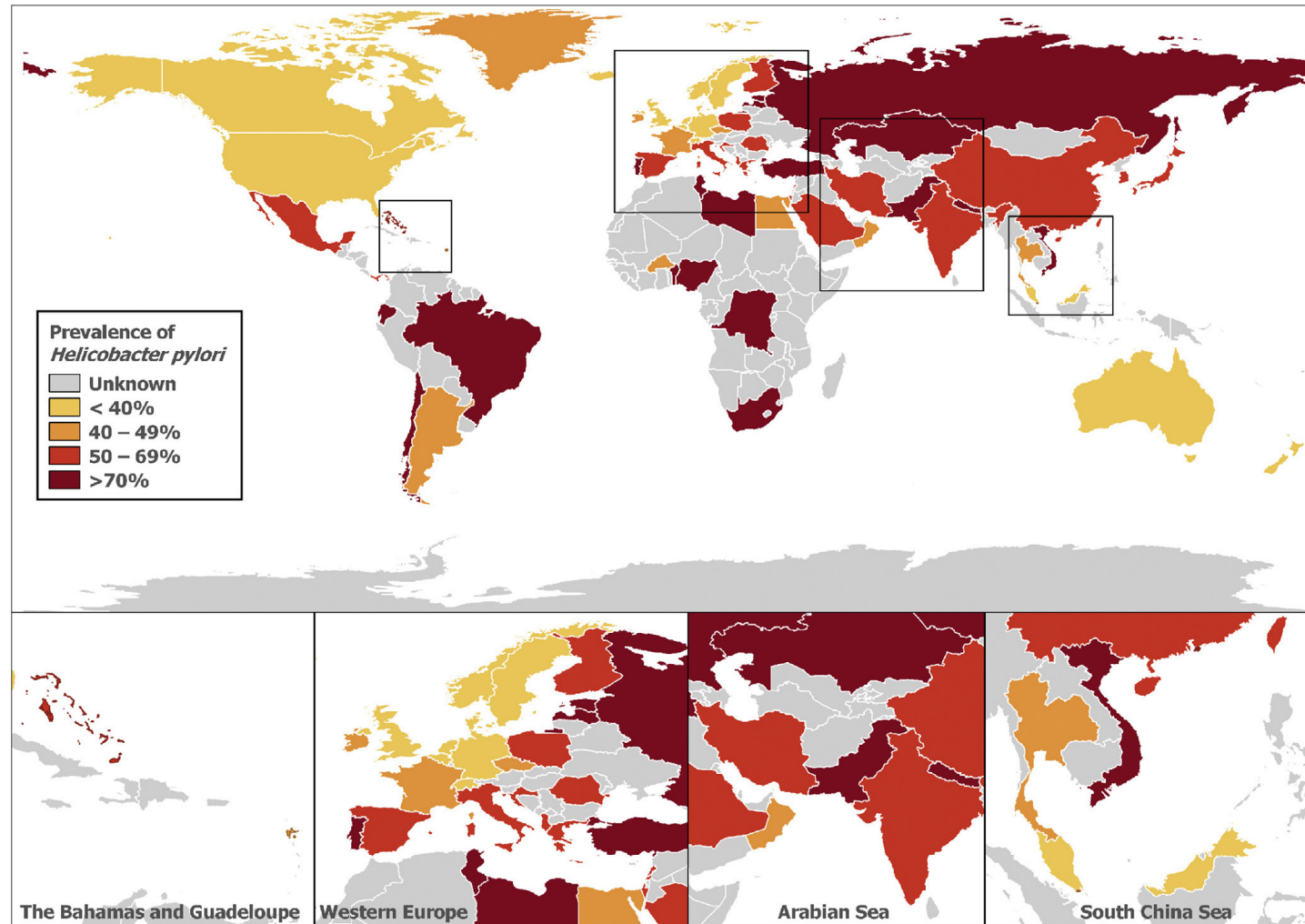
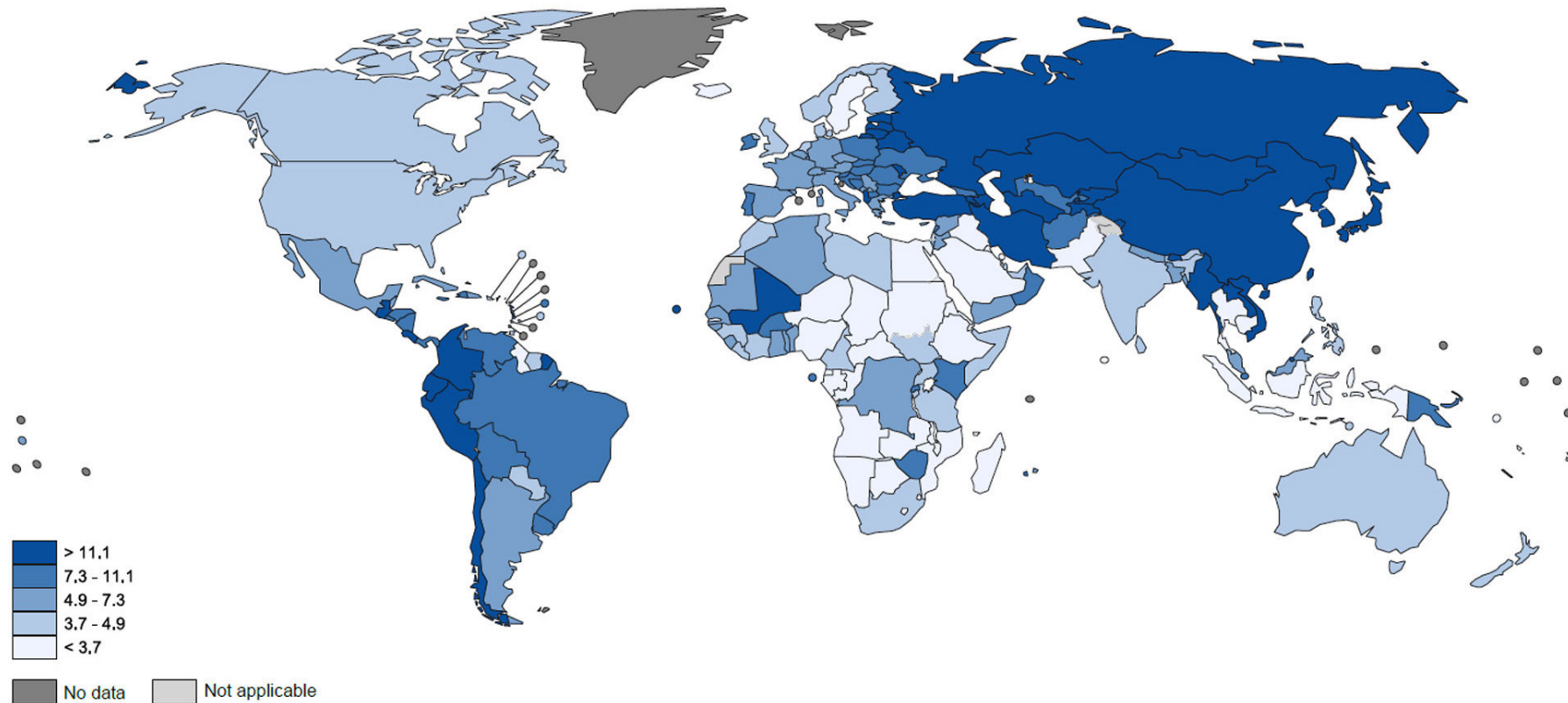


Figure 2. Global prevalence of HP choropleth map. Certain regions are magnified to better display the smaller countries. The online interactive global map showing the HP prevalence can be found at the following URL: <https://people.ucalgary.ca/~ggkaplan/HP2016.html>.

JKY Hooi, *Gastroenterology* 153, 420-429, 2017

Worldwide prevalence of Gastric Cancer Patients



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data source: Globocan 2018
Map production: CSU
World Health Organization

 **World Health Organization**
© WHO 2018. All rights reserved

Figure 5.

Gastric cancer: Estimated age-standardized incidence rates per 100,000 person-years in 2018. Source: GLOBOCAN 2018

M Arnold, *Gastroenterology* 159, 335-349, 2020

Treatment of *H. pylori* infection

1st-line therapy

- Penicillin antibiotic : Amoxicillin
- Macrolide antibiotic : Clarithromycin
- Proton pump inhibitor (PPI)

Successful eradication
(About 70 %)

Relapse · Exacerbation
(About 10 %)

Morgan DR et al., LMMA., 2013

About 30 %

2nd-line therapy

- Penicillin antibiotic : Amoxicillin
- Imidazole antibiotics : Metronidazole
- Proton pump inhibitor (PPI)

Successful eradication
(About 90 %)

About 10 %

3rd-line therapy

- Therapy regimen is not defined.
- Penicillin antibiotic : Amoxicillin
 - New Quinolone antibiotics : Levofloxacin
 - Proton pump inhibitor (PPI)

Successful eradication
(About 95 %)

Eradication failure
(About 5 %)

Gisbert JP et al., Am J Gastroenterol, 2006

Problems

- Up to 50 % of patients have side effects while taking *H. pylori* treatment.
- PPI metabolism and drug resistance leads to treatment failure.



Anti-*H. pylori* Activities of Intervenolin derivatives

H. pylori

Antibiotics

Strain		Intervenolin	AS-1664	AS-1934	Clarithromycin	Amoxicillin	Metronidazole
<i>Helicobacter pylori</i>	JCM 12093	0.0156	0.0312	0.125	0.0156	0.0078	0.25
<i>Helicobacter pylori</i>	JCM 12095	0.0156	0.0312	0.25	0.0156	0.0156	ND
<i>Helicobacter pylori</i>	SS-1	0.125	0.0312	0.125	0.0312	0.125	ND
<i>Helicobacter pylori</i>	ATCC 49503	0.125	0.0312	0.125	0.0156	0.0078	8
<i>Helicobacter pylori</i>	ATCC 700684 (CAM-R)	0.25	0.0156	0.125	64	0.0625	1
<i>Helicobacter pylori</i>	ATCC 43504 (MNZ-R)	1	0.0625	0.5	0.0625	0.0312	64



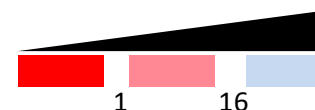
Ohishi, T et al, *Helicobacter* 23, e12470 (2018)

Anti-Microbial Activities of Intervenolin derivatives

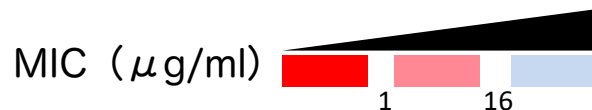
Common bacteria			Intervenolin	AS-1664	AS-1934	Clarithromycin	Amoxicillin
<i>Staphylococcus aureus</i>	FDA 209P		>64	>64	>128	0.125	0.0625
<i>Staphylococcus aureus</i>	Smith		128	>64	>128	0.125	0.0625
<i>Staphylococcus aureus</i>	MS9610		128	>64	>128	>128	8
<i>Staphylococcus aureus</i>	MRSA No.5		128	>64	>128	>128	128
<i>Staphylococcus aureus</i>	TY-04282(MRSA)		>128	>64	>128	>128	64
<i>Staphylococcus aureus</i>	Mu50		>128	>64	>128	>128	16
<i>Micrococcus luteus</i>	FDA 16		16	32	>64	0.063	0.125
<i>Micrococcus luteus</i>	IFO 3333		16	64	>64	0.063	0.125
<i>Micrococcus luteus</i>	PCI 1001		16	32	>64	<0.031	<0.0312
<i>Bacillus subtilis</i>	NRRL B-558		16	>64	>128	0.125	<0.0312
<i>Bacillus subtilis</i>	PCI 219		64	>64	>128	0.125	<0.0312
<i>Bacillus cereus</i>	ATCC 10702		>64	>64	>128	0.25	2
<i>Corynebacterium bovis</i>	1810		16	>64	>64	<0.031	0.25
<i>Enterococcus faecalis</i>	JCM 5803		>64	>64	>128	0.5	0.5
<i>Enterococcus faecalis</i>	NCTC12201		>64	>64	>128	128	1
<i>Enterococcus faecalis</i>	NCTC12203		>64	>64	>128	128	1
<i>Enterococcus faecium</i>	JCM 5804		>64	>64	>128	2	0.5
<i>Enterococcus faecium</i>	NCTC12202		>64	>64	>128	128	16
<i>Enterococcus faecium</i>	NCTC12204		>64	>64	>64	>64	16
<i>Escherichia coli</i>	NIHJ		>64	>64	>64	16	0.5
<i>Escherichia coli</i>	K-12		>64	>64	>128	32	2
<i>Escherichia coli</i>	K-12 ML1629		>64	>64	>64	64	8
<i>Escherichia coli</i>	BEM11		>64	>64	>64	1	4
<i>Escherichia coli</i>	BE1121		>64	>64	>64	1	4
<i>Escherichia coli</i>	BE1186		>64	>64	>128	2	1
<i>Shigella dysenteriae</i>	JS11910		>64	>64	>64	16	0.5
<i>Salmonella enteritidis</i>	1891		>64	>64	>64	4	0.5
<i>Proteus vulgaris</i>	OX19		>64	>64	>64	>64	0.5
<i>Proteus mirabilis</i>	IFM OM-9		>64	>64	>128	>64	0.25
<i>Serratia marcescens</i>	B-0524		>64	>64	>64	64	128
<i>Pseudomonas aeruginosa</i>	A3		>64	>64	>64	16	>128
<i>Klebsiella pneumoniae</i>	PCI 602		>64	>64	>64	4	32
<i>Candida albicans</i>	3147		>64	>64	>64	32	>128
<i>Mycobacterium smegmatis</i>	ATCC607*		>64	>64	128	0.5	>128

Ohishi, T et al, Helicobacter 23, e12470 (2018)

MIC ($\mu\text{g/ml}$)



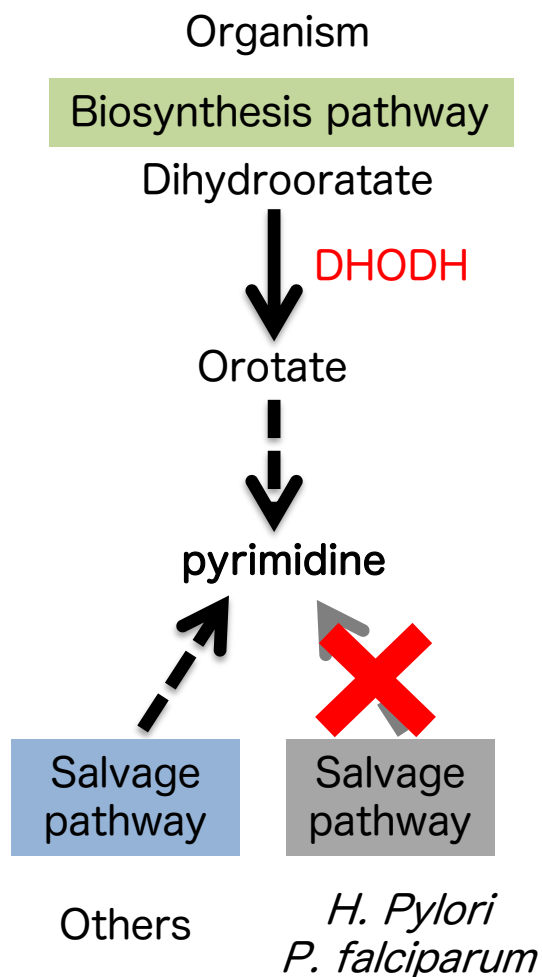
Anti-Intestinal Bacterial Activities of Intervenolin derivatives



Intestinal bacteria			Intervenolin	AS-1664	AS-1934	Clarithromycin	Amoxicillin
<i>Bacteroides</i>	<i>distasonis</i>	JCM 5825	32	64	>64	0.25	1
<i>Bacteroides</i>	<i>fragilis merdae</i>	JCM 11019	>64	>64	>64	0.25	16
<i>Bacteroides</i>	<i>merdae</i>	JCM 9497	>64	64	>64	0.25	1
<i>Bacteroides</i>	<i>ovatus</i>	JCM 5824	>4	>64	>64	1	32
<i>Bacteroides</i>	<i>thetaiotaomicron</i>	JCM 5827	>64	>64	>64	1	32
<i>Bacteroides</i>	<i>uniformis</i>	JCM 5828	>64	>64	>64	0.5	2
<i>Bacteroides</i>	<i>vulgatus</i>	JCM 5826	>64	>64	>64	0.5	2
<i>Bacteroides</i>	<i>eggerthii</i>	JCM 12986T	>64	>64	>64	0.5	0.125
<i>Bifidobacterium</i>	<i>adolescentis</i>	JCM 1251	>64	>64	>64	<0.0312	0.125
<i>Bifidobacterium</i>	<i>angulatum</i>	JCM 7096	>64	>64	>64	0.0625	0.25
<i>Bifidobacterium</i>	<i>bifidum</i>	JCM 1209	>64	>64	>64	0.5	0.5
<i>Bifidobacterium</i>	<i>breve</i>	JCM 1192	>64	>64	>64	<0.0312	0.5
<i>Bifidobacterium</i>	<i>infantis</i>	JCM 1222	16	>64	>64	<0.0312	0.25
<i>Collinsella</i>	<i>aerofaciens</i>	JCM 10188	>64	>64	>64	<0.0312	0.125
<i>Eggerthella</i>	<i>lenta</i>	JCM 9979	64	>64	>64	<0.0312	1
<i>Eubacterium</i>	<i>limosum</i>	JCM 6421	8	>64	64	<0.0312	0.25
<i>Eubacterium</i>	<i>rectale</i>	JCM 17463	64	64	>128	16	0.25
<i>Fusobacterium</i>	<i>varium</i>	JCM 6320T	>64	>64	>64	>64	2
<i>Lactobacillus</i>	<i>acidophilus</i>	JCM 1132	>64	>64	>64	0.0625	0.5
<i>Lactobacillus</i>	<i>fermentum</i>	JCM 1173	>64	>64	>64	0.0625	0.125
<i>Lactobacillus</i>	<i>gasseri</i>	JCM 1131	>64	>64	>64	0.0625	0.125
<i>Lactobacillus</i>	<i>plantarum</i>	JCM 1149	>64	>64	>64	0.125	0.125
<i>Megasphaera</i>	<i>elsdenii</i>	JCM 1772T	32	>64	>64	0.0625	0.25
<i>Peptostreptococcus</i>	<i>anaerobius</i>	JCM 1769	32	>64	>64	0.0625	0.25
<i>Blautia (Ruminococcus)</i>	<i>productus</i>	JCM 1471T	16	>64	>64	0.125	0.5
<i>Blautia (Ruminococcus)</i>	<i>hydrogenotrophicus</i>	JCM 14656T	8	>64	>64	0.125	0.25
<i>Blautia (Ruminococcus)</i>	<i>hansenii</i>	JCM 14655T	8	>64	64	0.0625	1
<i>Veillonella</i>	<i>parvula</i>	JCM 12972T	>64	>64	>64	8	0.25
<i>Enterococcus</i>	<i>faecalis</i>	JCM 5803	>128	>128	>128	1	0.5
<i>Enterococcus</i>	<i>faecalis</i>	NCTC12201	>128	>128	>128	128	0.5
<i>Enterococcus</i>	<i>faecalis</i>	NCTC12203	>128	>128	>128	128	0.5
<i>Enterococcus</i>	<i>faecium</i>	JCM 5804	>128	>128	>128	2	0.25
<i>Enterococcus</i>	<i>faecium</i>	NCTC12202	>128	>128	>128	128	8
<i>Enterococcus</i>	<i>faecium</i>	NCTC12204	>128	>128	>128	128	8
<i>Clostridium</i>	<i>bifementans</i>	JCM 1386T	16	>64	>64	0.125	0.25
<i>Clostridium</i>	<i>butyricum</i>	JCM 1391T	64	>64	>64	0.0625	0.125
<i>Blautia (Clostridium)</i>	<i>coccoides</i>	JCM 1395T	16	>64	64	0.125	0.5
<i>Clostridium</i>	<i>difficile</i>	JCM 1296	>64	>64	>64	0.25	2
<i>Clostridium</i>	<i>difficile</i>	BAA-1382	64	>64	>64	0.5	4
<i>Clostridium</i>	<i>ndolis</i>	JCM 1380T	32	>64	>64	0.25	1
<i>Clostridium</i>	<i>innocuum</i>	JCM 1292T	64	>64	>64	0.25	0.5
<i>Clostridium</i>	<i>limosum</i>	JCM 1427T	32	>64	>64	0.125	<0.0312
<i>Clostridium</i>	<i>perfrengens</i>	PB6K	32	>64	>64	0.5	<0.0312
<i>Clostridium</i>	<i>ramosum</i>	JCM 1298T	>64	>64	>64	0.25	0.25
<i>Escherichia</i>	<i>coli</i>	K-12	>64	>64	>64	16	2
<i>Klebsiella</i>	<i>pneumoniae</i>	PCI602	>64	>64	>64	8	8
<i>Klebsiella</i>	<i>oxytoca</i>	GN17031	>64	>64	>64	>64	>182

Ohishi, T et al, Helicobacter 23, e12470 (2018)

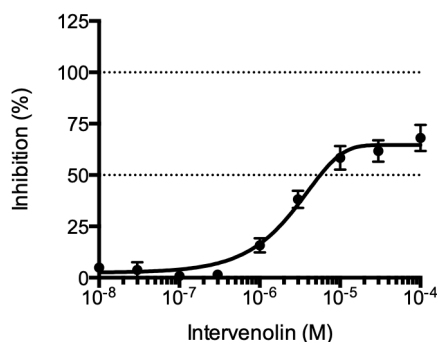
Effect of Intervenolin Derivatives on DHODH



DHODH: dihydroorotate dehydrogenase

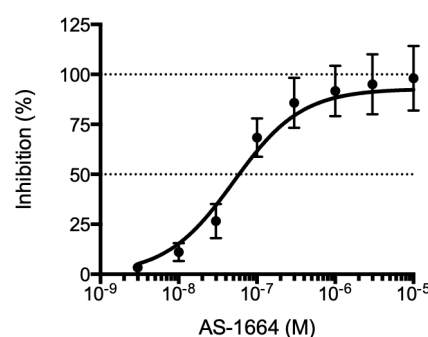
Intervenolin

$$IC_{50} = 11.11 \pm 2.44 \mu M$$



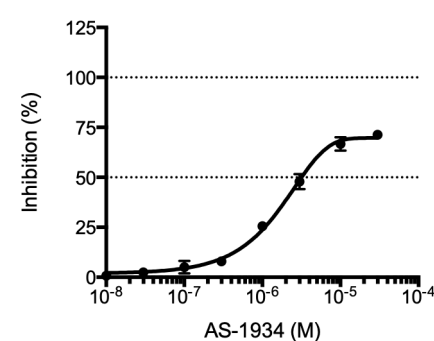
AS-1664

$$IC_{50} = 0.06 \pm 0.02 \mu M$$



AS-1934

$$IC_{50} = 4.55 \pm 0.49 \mu M$$



Ohishi, T. et al, Helicobacter 23, e12470 (2018)

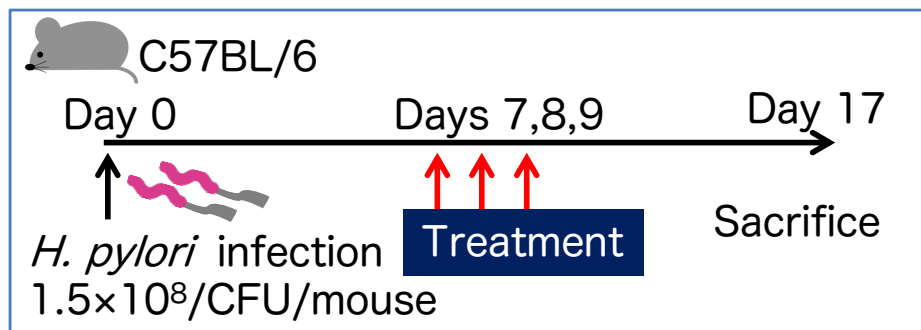
Triple Therapy of *H. pylori*

- Proton pump inhibitor (PPI) (Omeprazole) 20 mg/shot
- Penicillin antibiotic : Amoxicillin 750 mg/shot
- Macrolide antibiotic : Clarithromycin 200 mg/shot

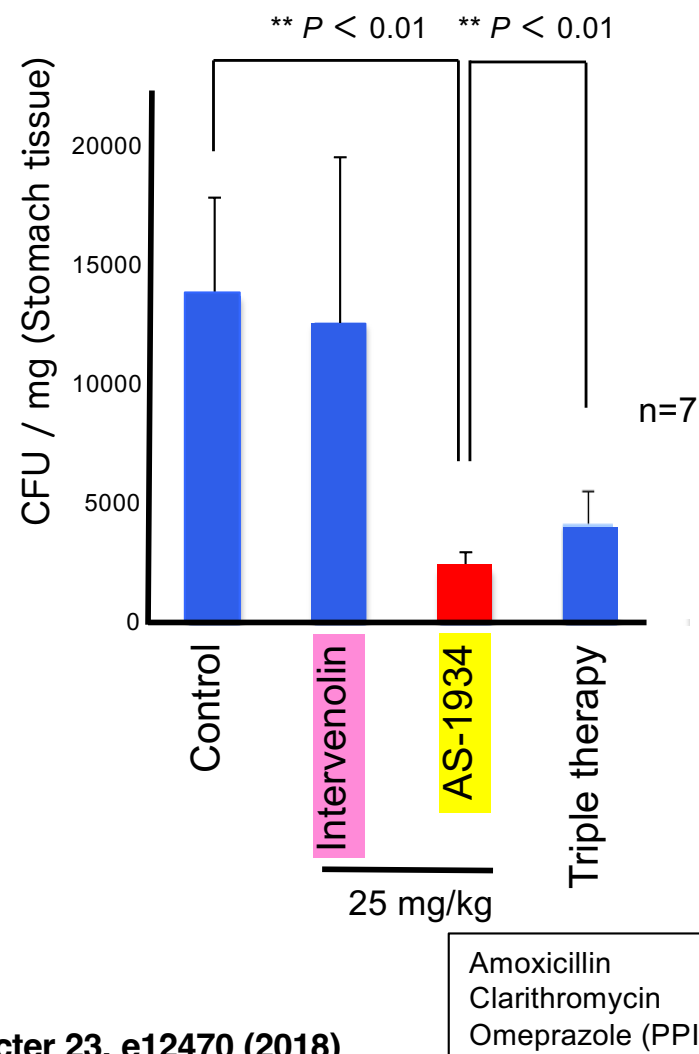
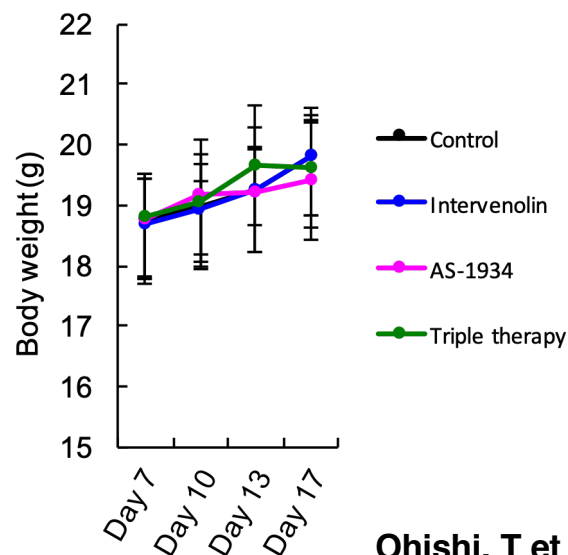
Twice a day for 7 days



Anti-*H. pylori* Activities of Intervenolin derivatives *in vivo*



Triple therapy: omeprazole 400 μ mol/kg, after 30 min amoxicillin 28.5 mg/kg+clarithromycin 14.3 mg/kg

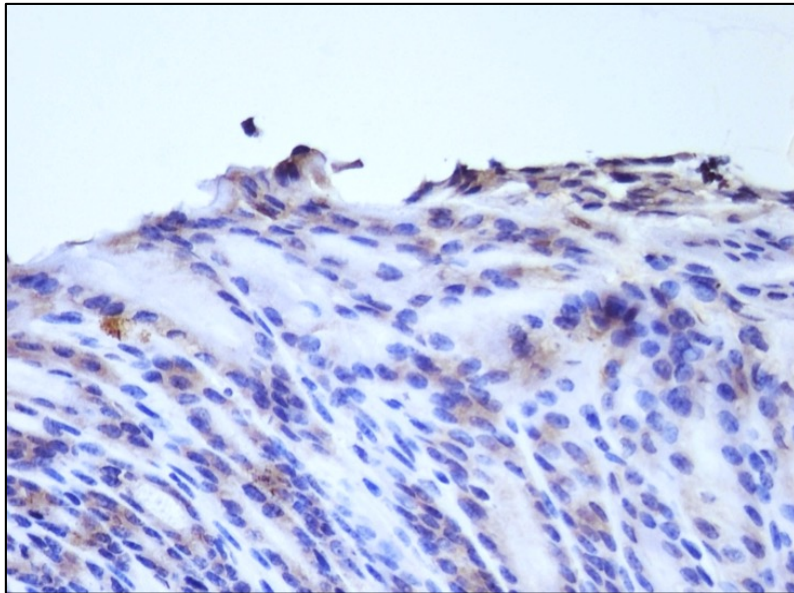


Ohishi, T et al, *Helicobacter* 23, e12470 (2018)

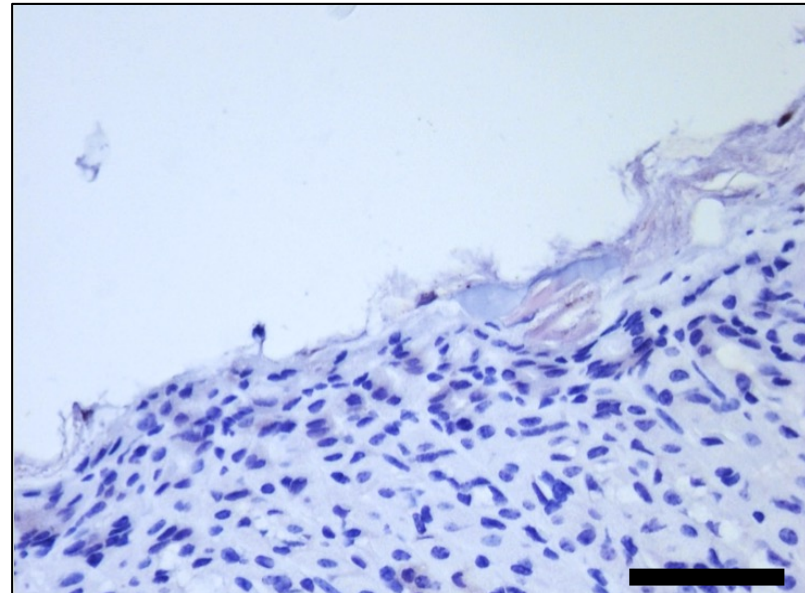
Effect of AS-1934 on *H. pylori* in vivo

IHC: anti-*H. pylori*

Control



AS-1934



Bar: 50 μ m

AS-1934 is Stable under Acidic Condition



<http://biorelevant.com>

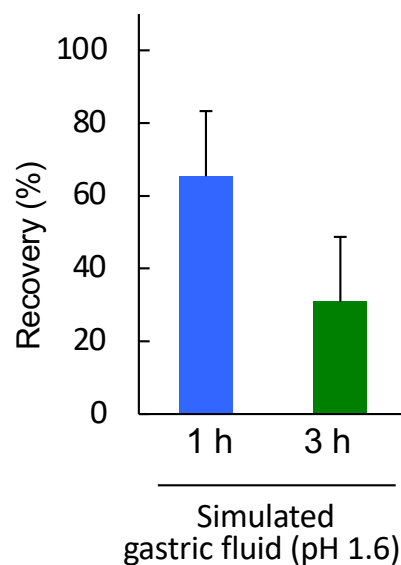
Table I. Sample Composition for Simulating Fasted State Gastric Conditions (FaSSGF)

FaSSGF pH 1.6	
Sodium taurocholate	80 μ M
Lecithin	20 μ M
Pepsin	0.1 mg/ml
NaCl	34.2 mM
HCl conc.	<i>qs ad</i> pH 1.6
Deionized water	<i>ad</i> 1 l
pH	1.6
Osmolality (mOsmol/kg)	120.7 $\pm$ 2.5
Buffer capacity (mEq/pH/L)	–
Surface tension (mN/m)	42.6

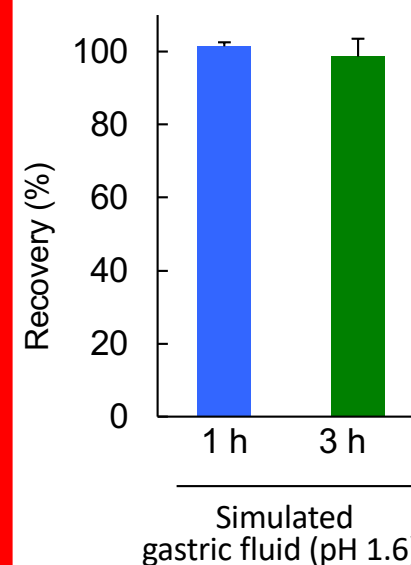
Klein S et al. AAPS J. , 2010

Simulated gastric fluid

Intervenolin

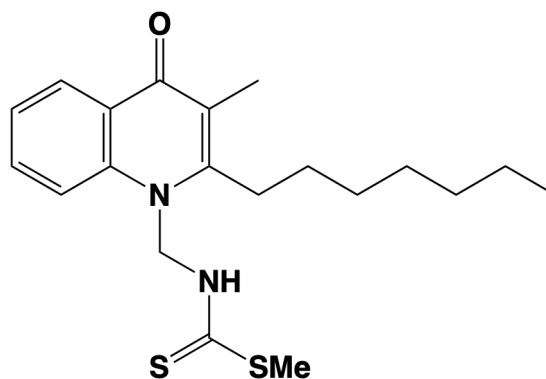


AS-1934



Ohishi, T et al, Helicobacter 23, e12470 (2018)

AS-1934, a Derivative of Intervenolin, Exerts Potent *Anti-H. pylori* Activity



AS-1934