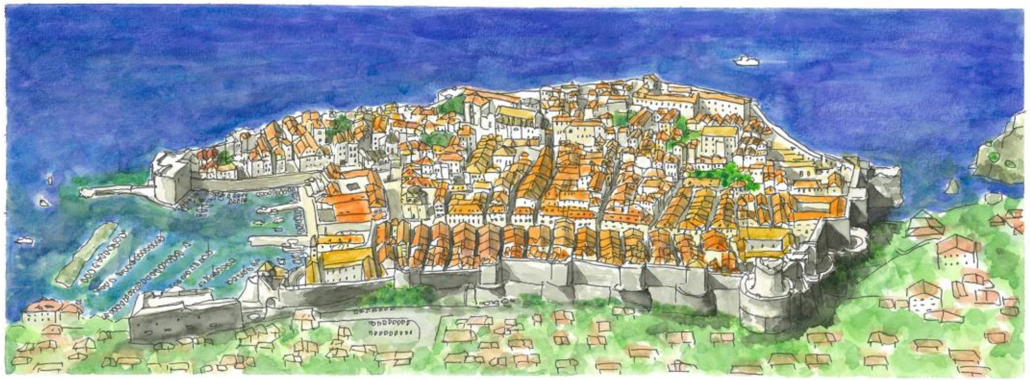
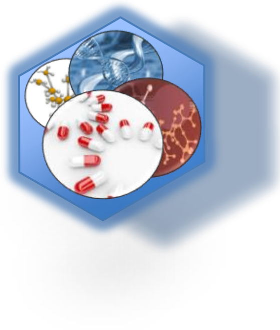
Modulators of the Tumor-Stromal Cell Interactions: New Candidates for Anti-tumor Drugs

Manabu Kawada

Laboratory of Oncology, Institute of Microbial Chemistry (BIKAKEN), Microbial Chemistry Research Foundation



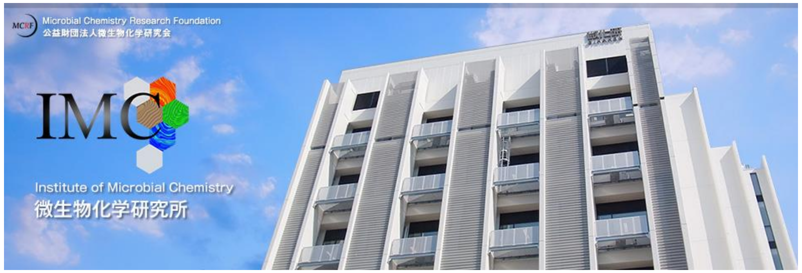


Institute of Microbial Chemistry (IMC), Microbial Chemistry Research Foundation (MCRF)

IMC Institute of Microbial Chemistry

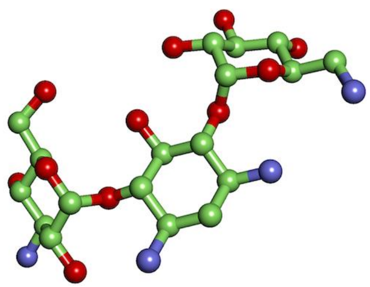
MCRF Microbial Chemistry Research Foundation

Microbial Chemistry Research Foundation
Institute of Microbial Chemistry
http://www.bikaken.or.jp/

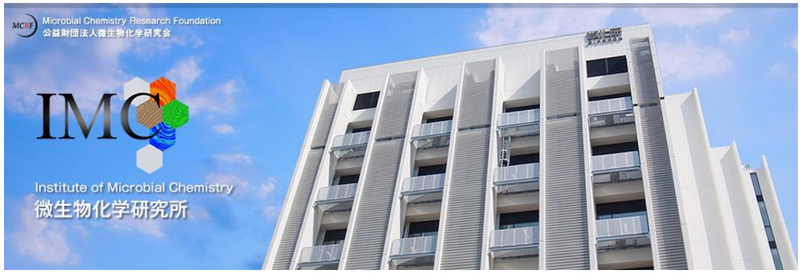


Tokyo, Headquarters

Prof. Hamao Umezawa founded in 1958.



Antibiotic
Kanamycin (1957）



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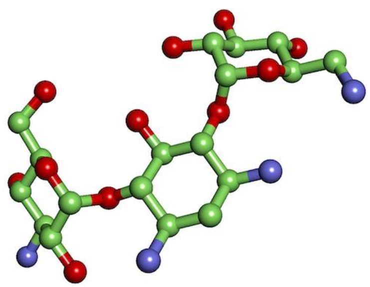

Antibiotic
Kanamycin (1957)

Microbial metabolites:
actinomycetes and fungus

New drugs:
antibiotics
antitumor drugs

MCRF

Drugs discovered and launched by MCRF

	Substance	Use	Discovered	Marketed	Production
1.	Kanamycin	Antibacterial drug	1957	1958	*Streptomyces kanamyceticus*
2.	Bekanamycin (Kanamycin B)	Antibacterial drug	1957	1969	*Streptomyces kanamyceticus*
3.	Bleomycin	Anticancer drug	1965	1969	*Streptomyces verticillus*
4.	Kasugamycin	Agrochemical for rice blast disease	1965	1970	*Streptomyces kasugaensis* M-338
5	Josamycin	Antibacterial drug	1967	1970	*Streptomyces narbonensis* var. josamyceticus A204-P/2
6.	Dibekacin (Panimycin)	Antibacterial drug	1971	1975	Synthesized from Bekanamycin
7.	Peplomycin	Anticancer drug	1978	1981	*Streptomyces verticillus* produces these compounds by precursor addition
8.	Aclarubicin	Anticancer drug	1975	1982	*Streptomyces galilaeus*
9.	Ubenimex (Bestatin)	Anticancer drug	1976	1987	*Streptomyces oliboreticuli* MD976-C7
10.	Pirarubicin	Anticancer drug	1979	1988	Synthesized from Daunorubicin
11.	Aivlosin	Antibacterial veterinary drug	1979	1988	Synthesized from Tylosin
12.	Arbekacin (Habekacin)	Antibacterial drug	1973	1990	Synthesized from Bekanamycin
13.	Gusperimus (Spanidin)	Immunosuppressive agent	1982	1990	*Bacillus laterosporus*
14.	Tildipirosin (Zuprevo)	Antibacterial veterinary drug	2005	2011	Synthesized from Tylosin

MCRF

Inhibitors discovered and distributed by MCRF

	Substance	Inhibitors	Discovered
1	Leupeptin	Serine/Cysteine proteinase	1969
2	Pepstatin	Aspartic proteinase	1970
3	Chymostatin	Serine/Cysteine proteinase	1970
4	Antipain	Serine/Cysteine proteinase	1972
5	Phosphoramidon	Metalloproteinase	1973
6	Elastatinal	Serine proteinase	1973
7	Amastatin	Aminopeptidase	1978
8	Ebelacton	Esterase, Lipase, Aminopeptidase	1980
9	Arphamenines	Aminopeptidase	1983
10	Diprotin A	Dipeptidylaminopeptidase	1984
11	Actinonin	Aminopeptidase	1985
12	Foroxymithine	Dipeptidylaminopeptidase	1985
13	Leuhistin	Aminopeptidase	1990
14	Cytostatin	Protein phosphatase 2A	1994
15	Heliquinomycin	DNA helicase	1996
16	Rubratoxin A	Protein phosphatase 2A	2010 (Activity)
17	Heparastatin (SF4)	Heparanase	1994

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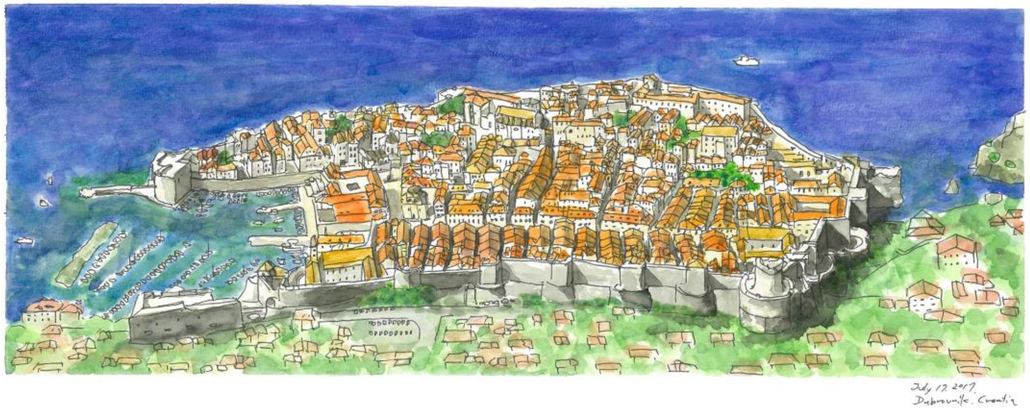
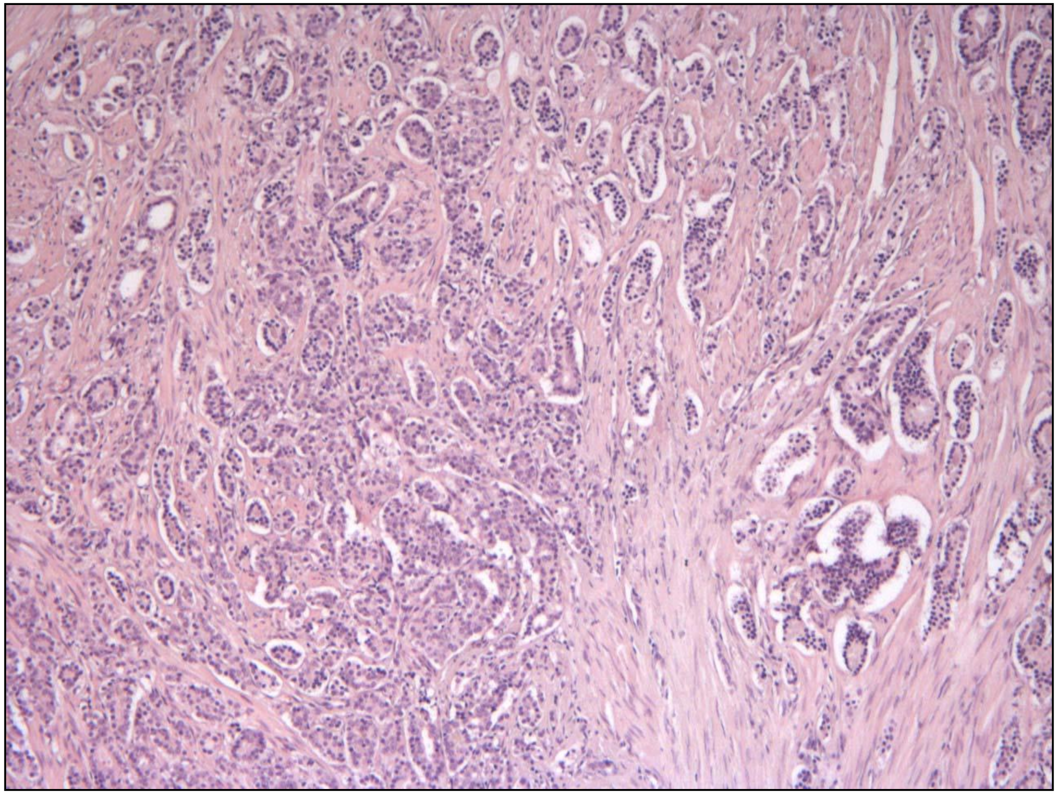


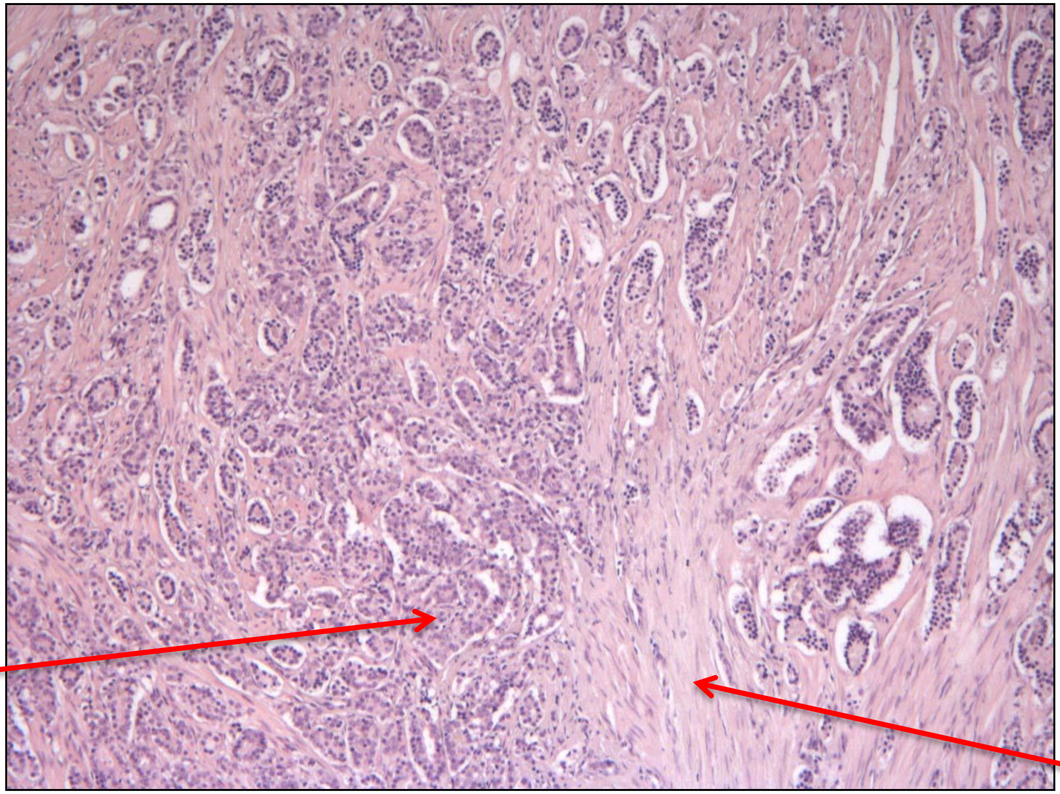
What are Tumor-stromal Cell Interactions？

Human Cancer Tissues Contain Lots of Stroma



Human prostate cancer tissue

Human Cancer Tissues Contain Lots of Stroma

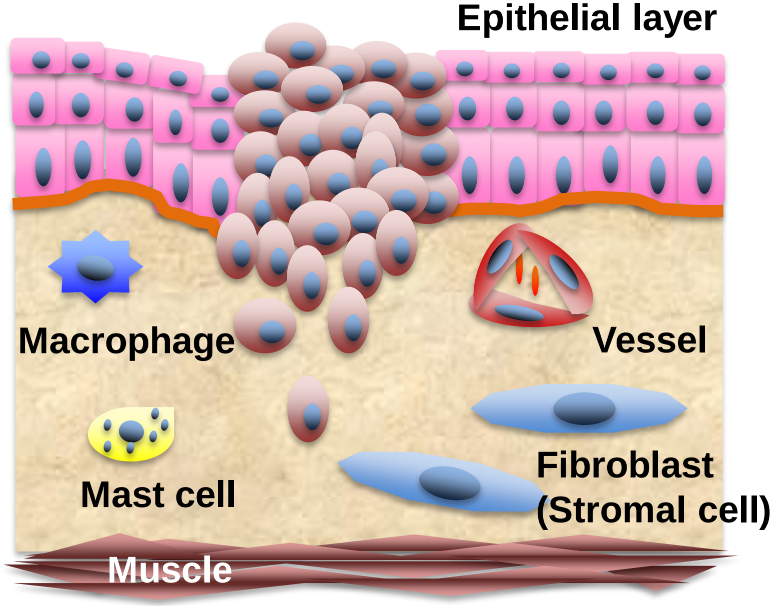


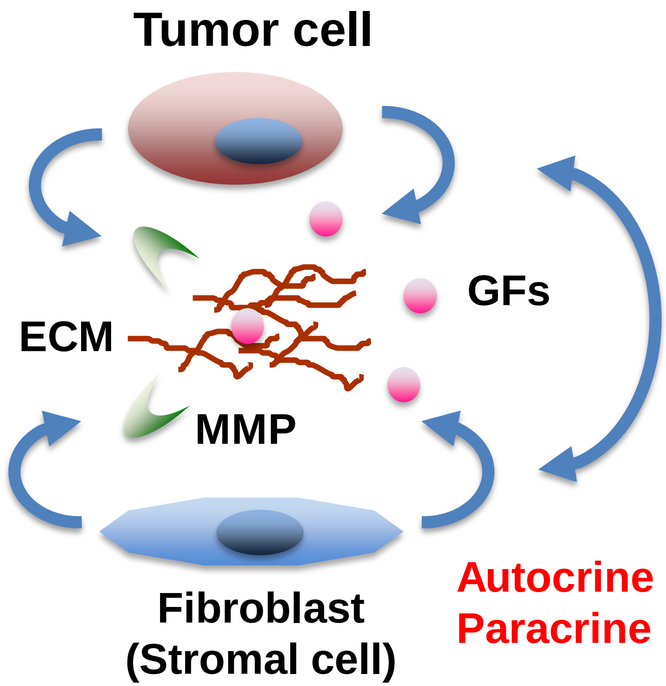
Cancer cells

Stroma

Human prostate cancer tissue

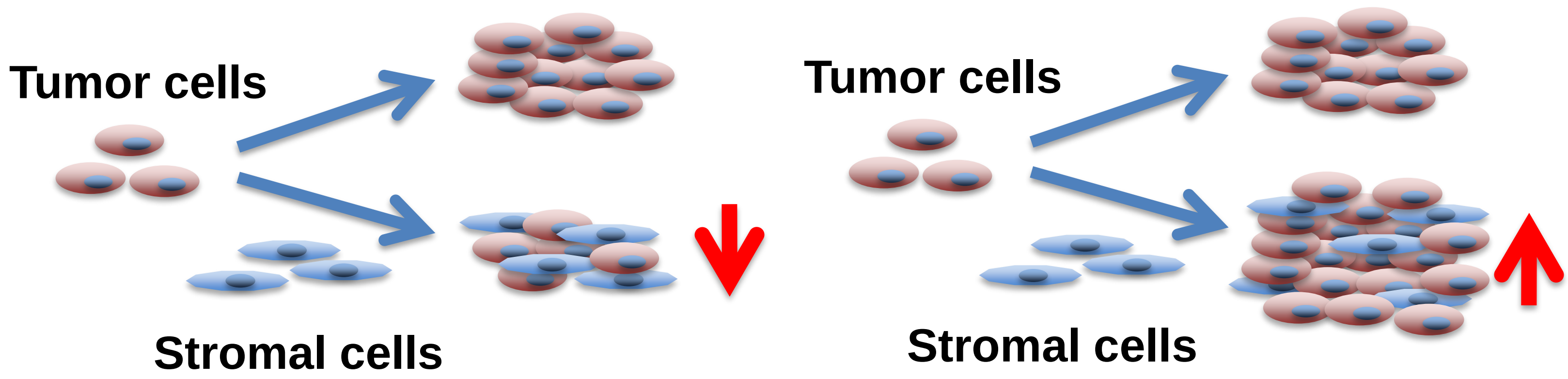
Modulation of Tumor-Stromal Cell Interactions

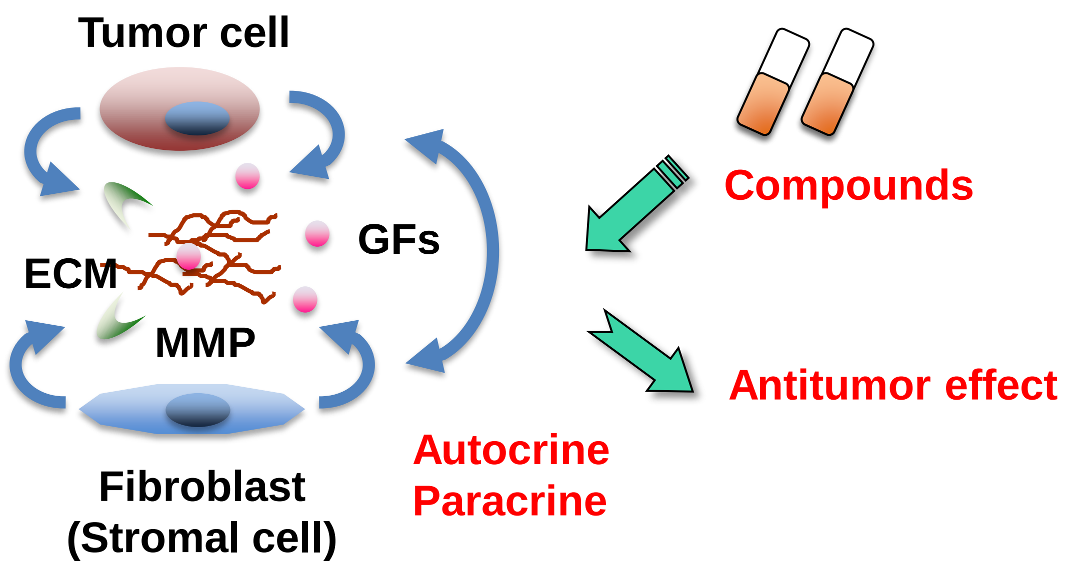




CAF (cancer-associated fibroblasts)

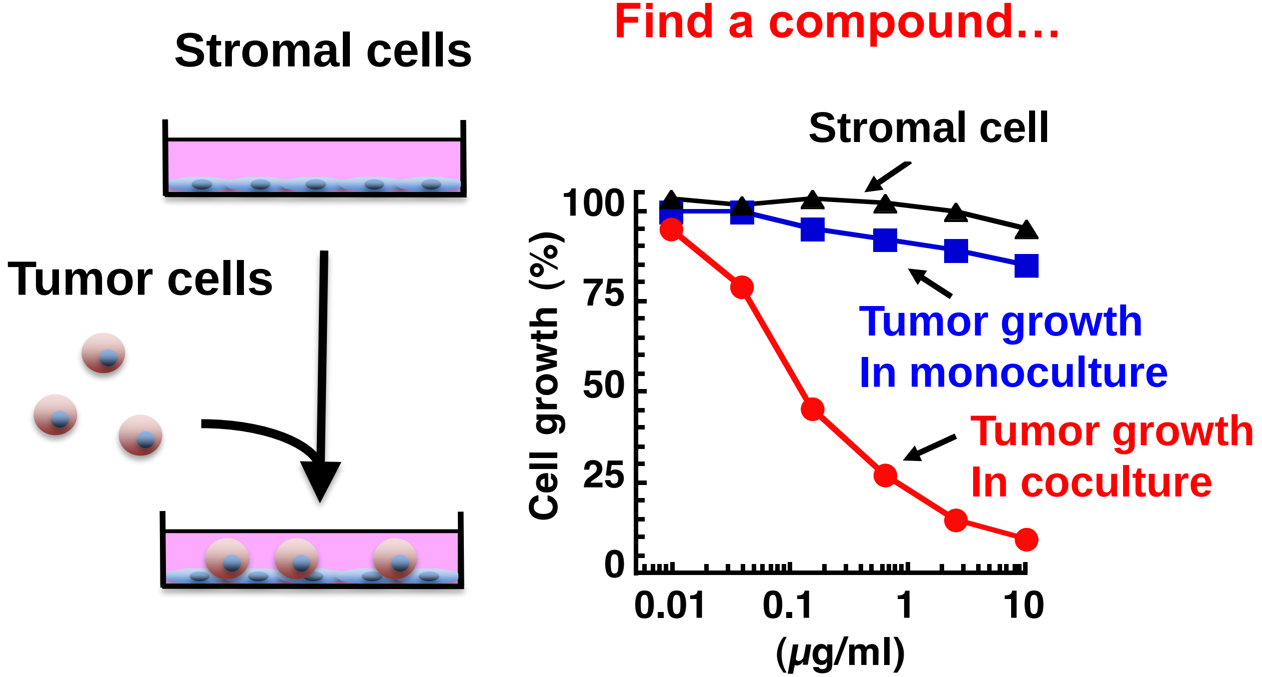
Stromal Cells Regulate Tumor Growth Positively and Negatively





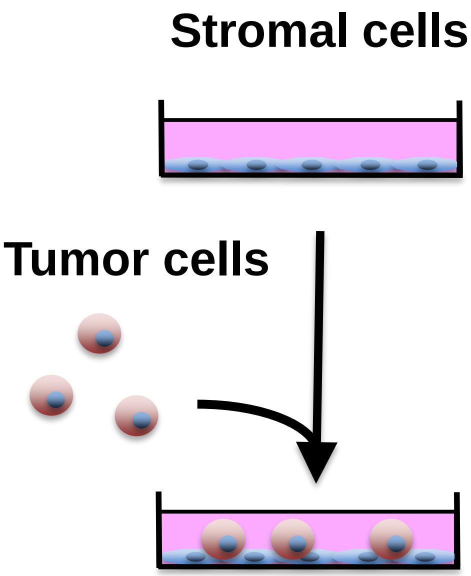
Coculture System for Screening of Modulators of Tumor-Stromal Cell Interactions

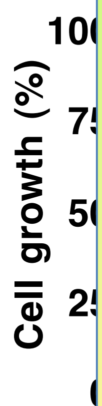
Find a compound…

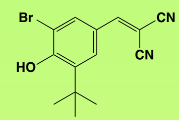


Kawada, M et al, Anticancer Res 24, 1561-1568 (2004)

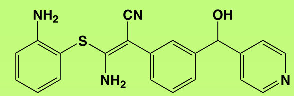
Coculture System for Screening of Modulators of Tumor-Stromal Cell Interactions



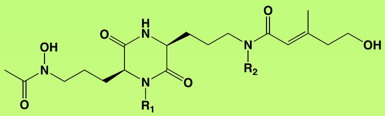




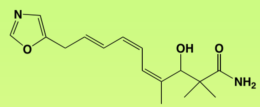
AG1024



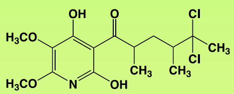
MEK inhibitor I



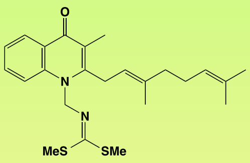
NBRI16716 A: R_1=OH, R_2=OH
NBRI16716 B: R_1=H, R_2=OH
NBRI16716 C: R_1=OH, R_2=H



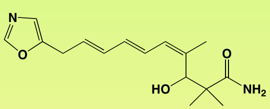
Phthoxazolin A



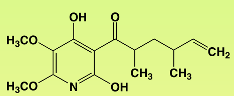
NBRI23477 A



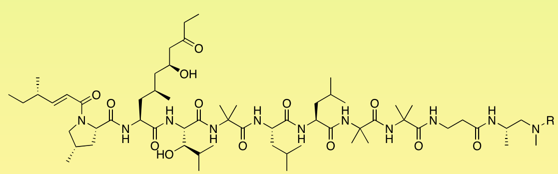
Intervenolin



Inthomycin B



NBRI23477 B

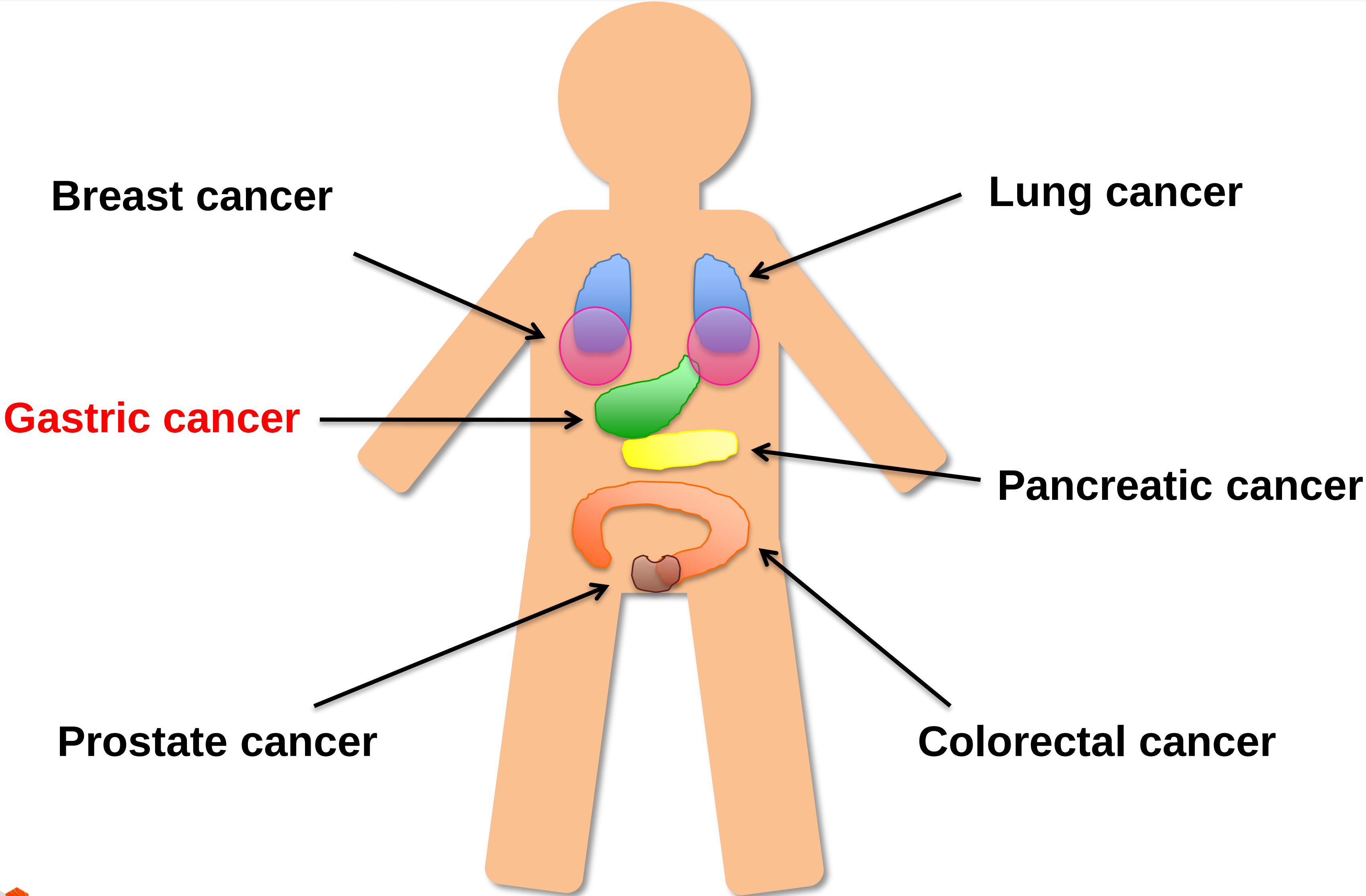


Leucinostatin A: R=CH_3
Leucinostatin B: R=H

Cancer Res 66, 4419-4425 (2006)
Cancer Sci 100, 150-157 (2009)
J Antibiot 62, 243-246 (2009)
J Antibiot 63, 319-323 (2010)
Int J Cancer 126, 810-818 (2010)
J Antibiot 66, 543-548 (2013)
PLoS ONE 10, e0119415 (2015)

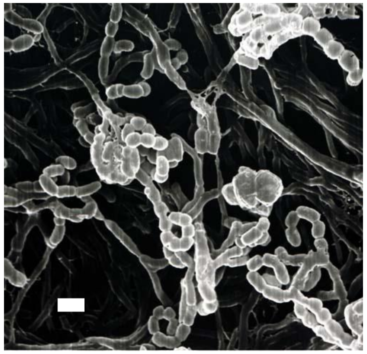
Kawada, M et a

Coculture Models of Various Cancers



Isolation of a New Compound from *Nocardia* sp. ML96-86F2

Nocardia sp. ML96-86F2



Bar=1 μm

Culture broth of *Nocardia* sp. ML96-86F2 10L

| filtration

mycelium

| MeOH ext/ filtration

filtrate

| EtOAc ext

Crude material (1 g)

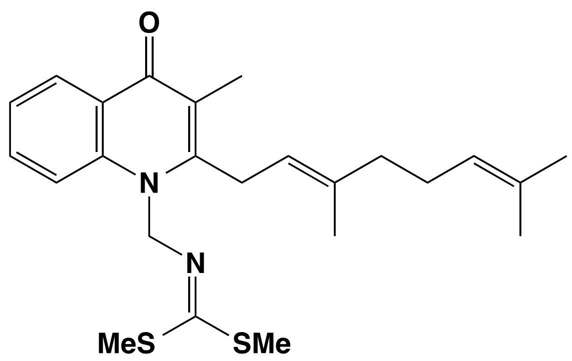
| Silica gel column, Hexane:EtOAc=1:1; 1:3, MeOH

H:E=1:1 fractions (58.3 mg)

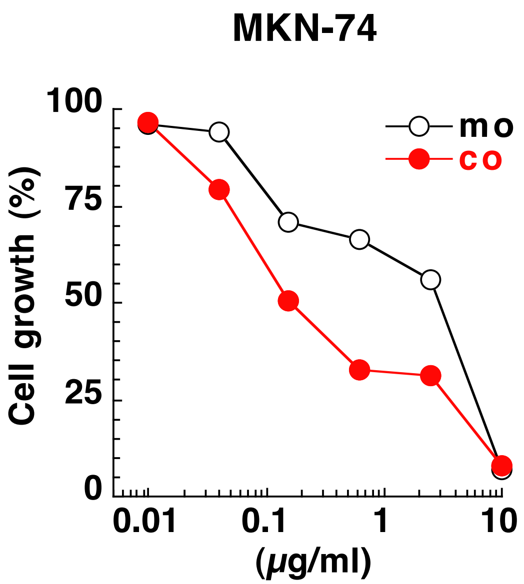
| HPLC 80%MeOH

New compound (3.9 mg)

Intervenolin, a New Compound, from *Nocardia* sp. ML96-86F2



Intervenolin

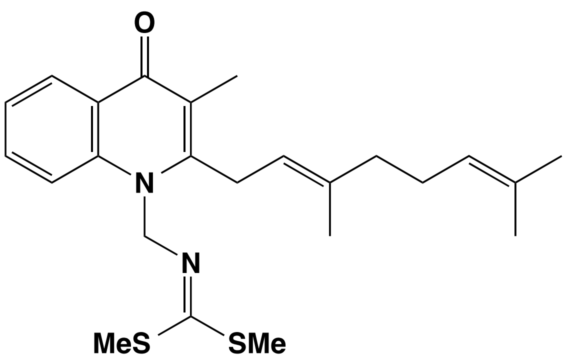


Kawada, M et al, J Antibiot 66, 543-548 (2013)

Physico-chemical properties of intervenolin

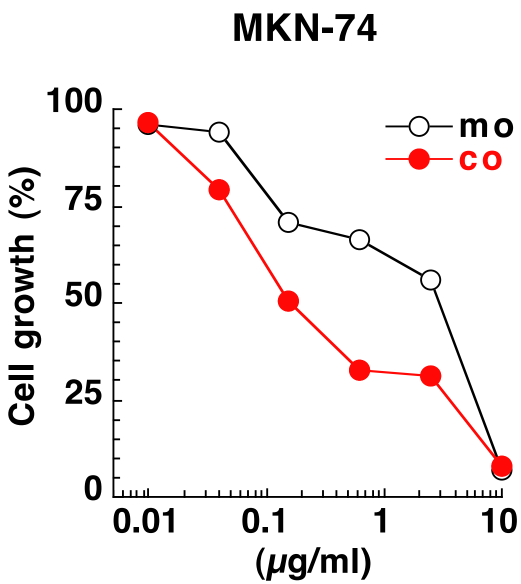
Appearance	Pale yellow oil
Molecular formula	$C_{24}H_{32}N_2OS_2$
HRESI-MS (*m/z*)	
Found	451.1834 $(M+Na)^+$
Calcd.	451.1848 for $C_{24}H_{32}N_2OS_2Na$
UVλ_{max} nm (ε) (MeOH)	214.5 (33,600), 242.5 (37,700), 327.5 (15,600), 341.0 (17,900)
IRυ_{max} (KBr) cm^{-1}	2966, 2921, 1617, 1596, 1562, 1372, 1281, 1193, 1022, 761, 696

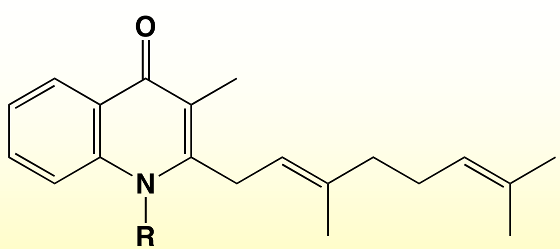
Intervenolin, a New Compound, from *Nocardia* sp. ML96-86F2



Intervenolin

Kawada, M et al, J Antibiot 66, 543-548 (2013)



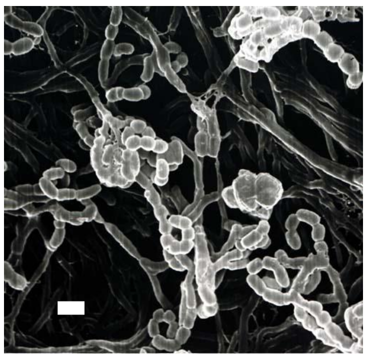


R=H CJ-13,136
R=CH_3 CJ-13,217

Anti-*Helicobacter pylori* activity
Dekker, KA et al, J Antibiot 51, 145-152 (1998)
Pfizer

Isolation of a New Compound from *Nocardia* sp. ML96-86F2

Nocardia sp. ML96-86F2



Bar=1 µm

<u>Culture broth of *Nocardia* sp. ML96-86F2 10L</u>

| filtration

mycelium

| MeOH ext/ filtration

filtrate

| EtOAc ext

Crude material (1 g)

| Silica gel column, Hexane:EtOAc=1:1; 1:3, MeOH

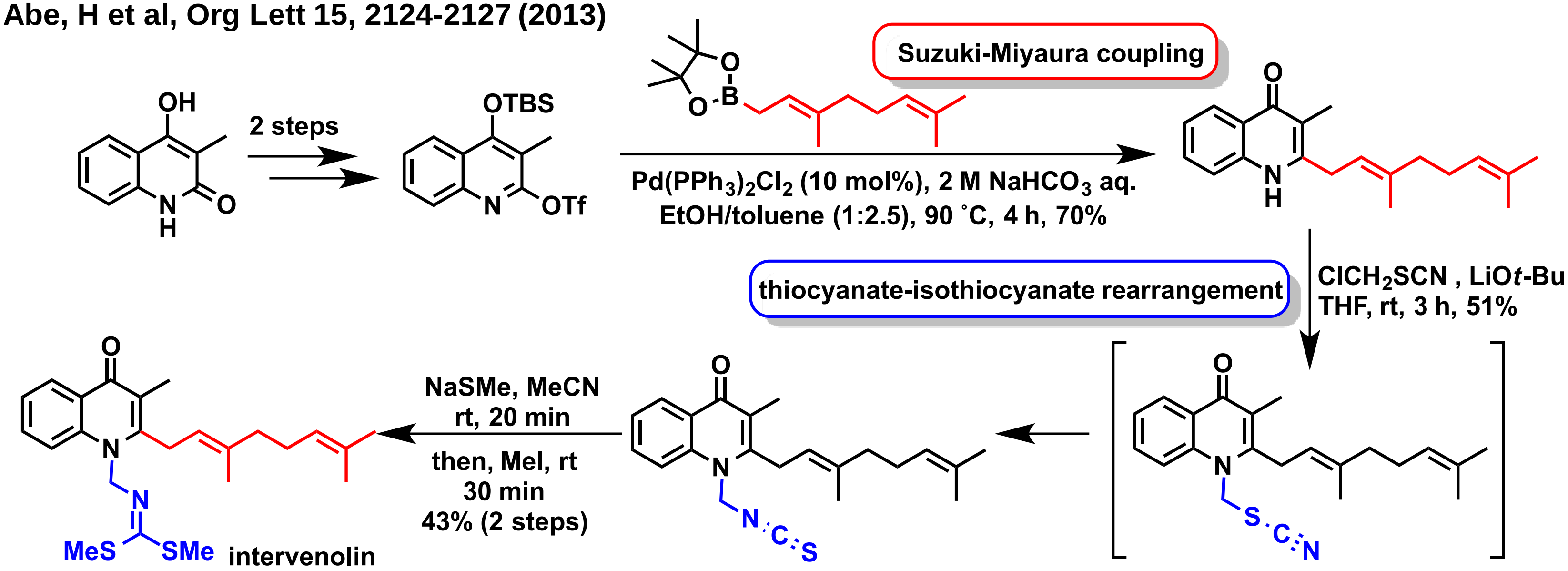
H:E=1:1 fractions (58.3 mg)

| HPLC 80%MeOH

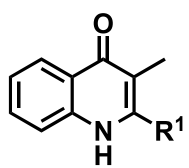
Intervenolin (3.9 mg)

Synthesis of Intervenolin and its Derivatives

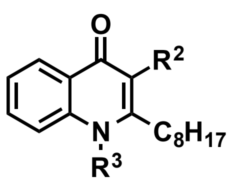
Abe, H et al, Org Lett 15, 2124-2127 (2013)



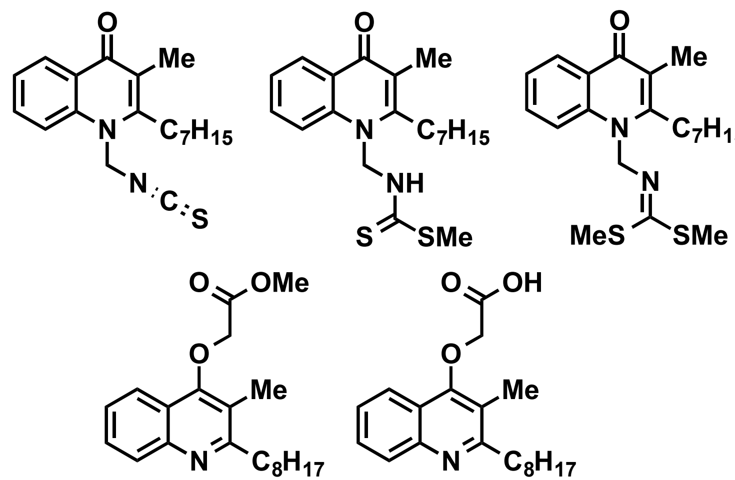
Abe, H et al, Tetrahedron 69, 7608-7617 (2013)



R^1 = C_3H_7, $CHMe_2$, C_5H_{11}, C_7H_{15}, C_8H_{17}, 4-F-Ph, C_2H_4Ph,

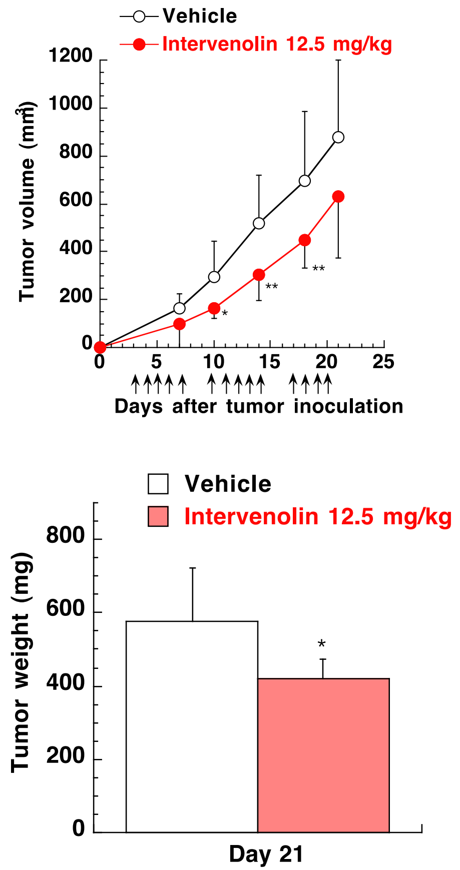


$R_2 = R_3 = H$
R_2 = Me, R_3 = Me, CN, CH_2CO_2Me, CH_2COSMe, CH_2CO_2H, $CH_2NHC(=O)SMe$

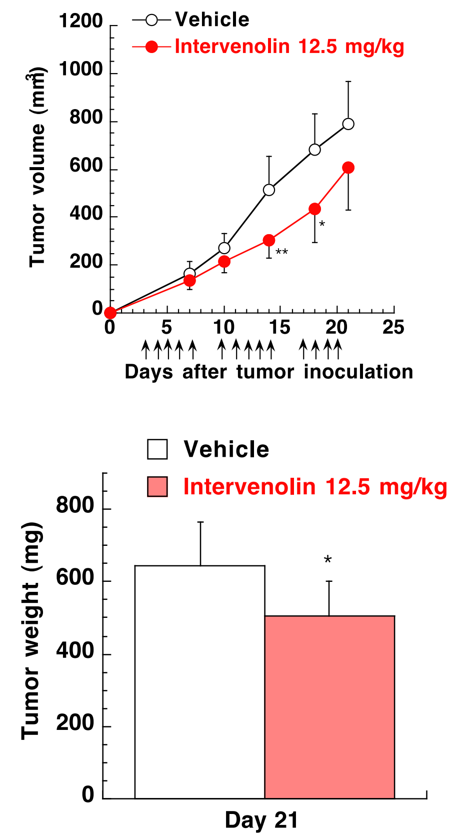


Effect of Intervenolin on Tumor Growth of MKN-74 Gastric Cancer Cells *in vivo*

MKN-74 tumor



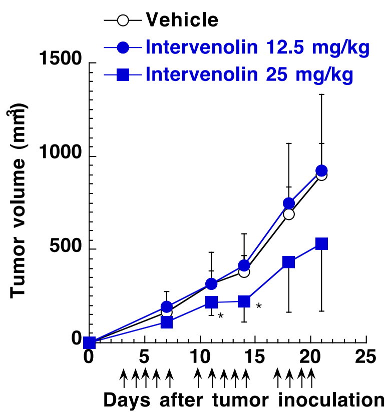
MKN-74+Hs738 tumor

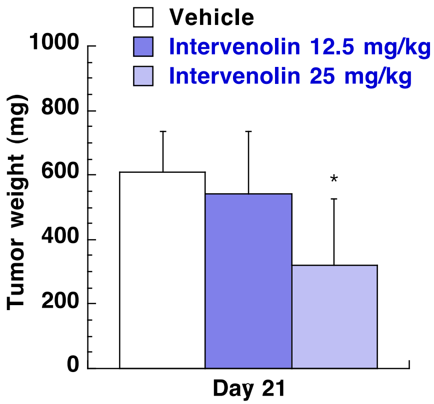


*P<0.05 and **P<0.01

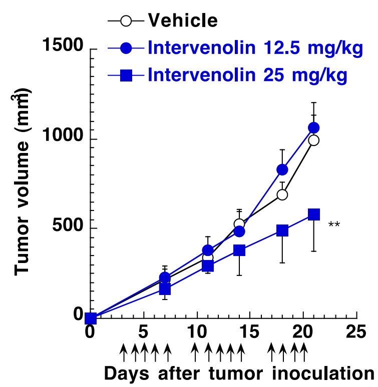
Effect of Intervenolin on Tumor Growth of HCT-15 Colorectal Cancer Cells *in vivo*

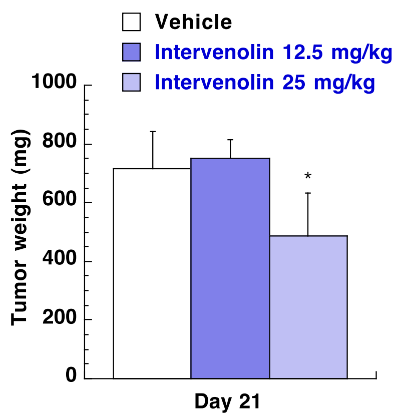
HCT-15 tumor



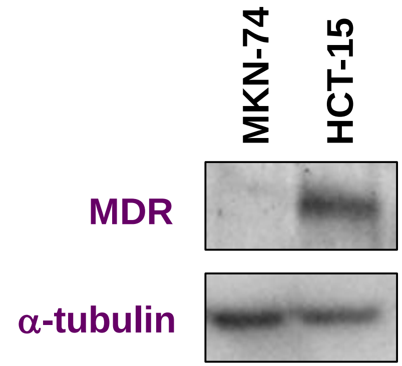


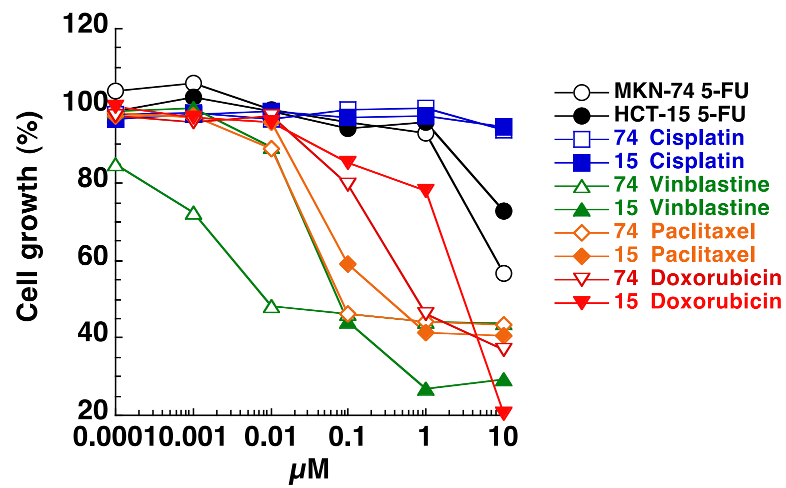
HCT-15 +CCD-18Co tumor





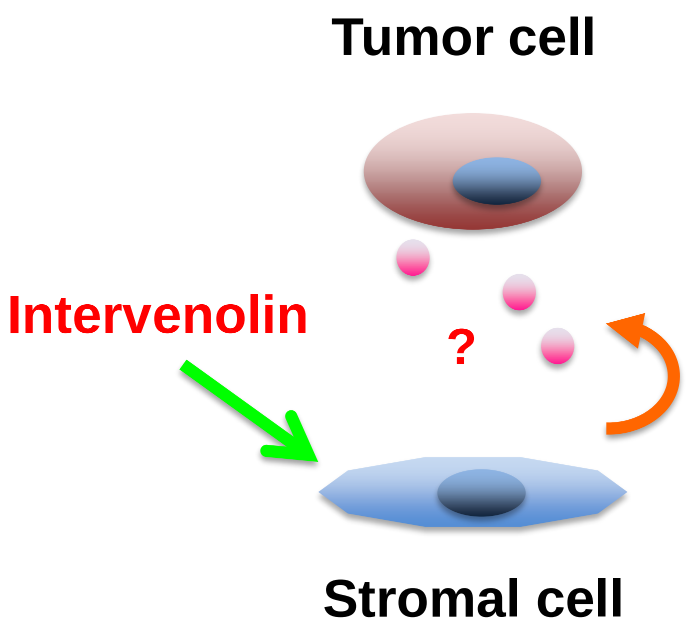
MDR expression





*P<0.05 and **P<0.01.

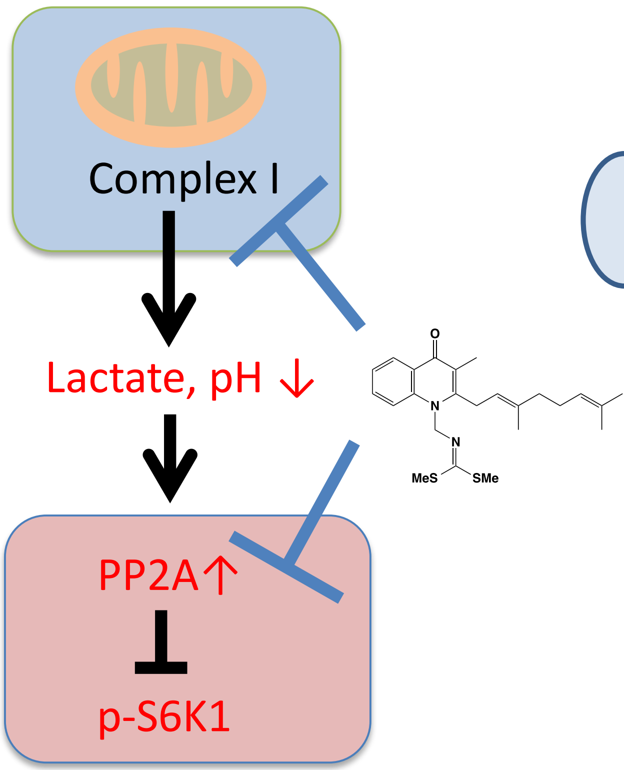
Possible Mechanism of Action of Intervenolin



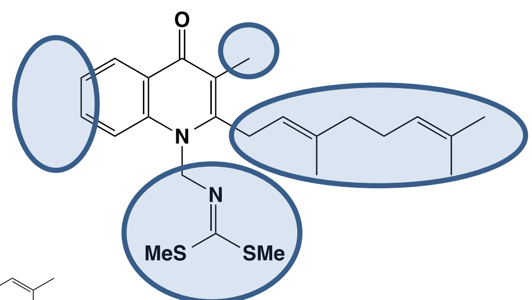
Development of Potent Intervenolin Derivatives

Mechanism of Action

Stromal cells



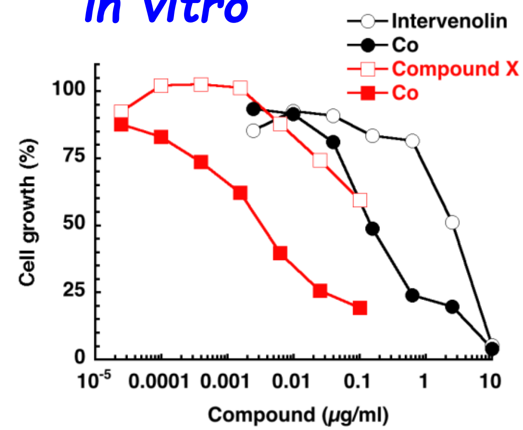
Tumor cells



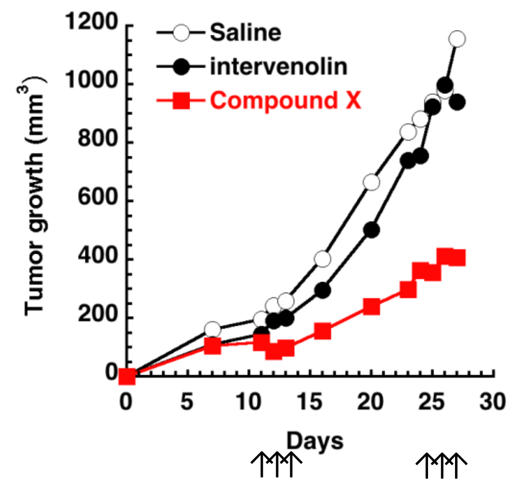
More than 100 derivatives

High potent derivatives

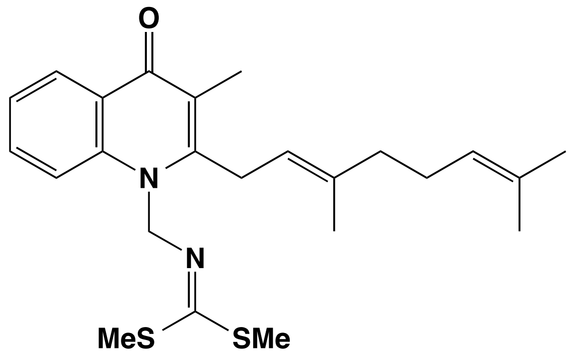
in vitro



in vivo

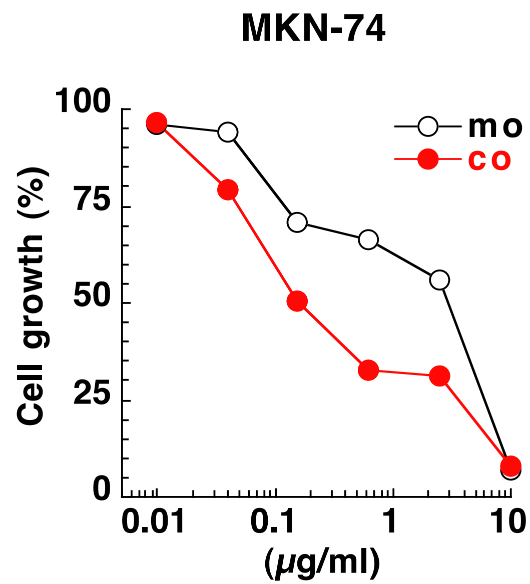


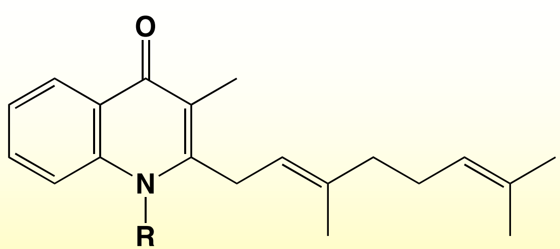
Intervenolin, a New Compound, from *Nocardia* sp. ML96-86F2



Intervenolin

Kawada, M et al, J Antibiot 66, 543-548 (2013)





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Anti-*Helicobacter pylori* activity
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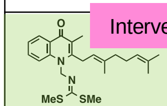
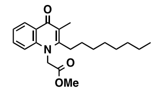
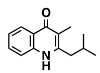
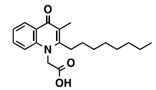
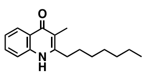
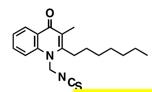
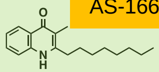
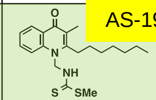
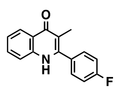
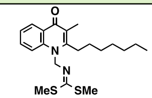
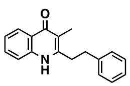
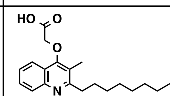
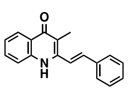
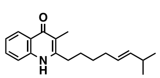
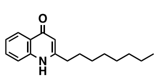
Pfizer

Anti-*H. pylori* Activities of Intervenolin derivatives

MIC (μg/ml)	*H. pylori* JCM 12093	*H. pylori* JCM 12095	*S. aureus* FDA209P	*E. faecalis* JCM5803	*E. coli* K-12	*H. influenzae* T-196	MTD (mg/kg)	MIC (μg/ml)	*H. pylori* JCM 12093	*H. pylori* JCM 12095	*S. aureus* FDA209P	*E. faecalis* JCM5803	*E. coli* K-12	*H. influenzae* T-196	MTD (mg/kg)
Intervenolin 	0.0156	0.0078	64	>128	>128	64	>50		2	2	>128	>128	>128	>128	>50
	0.5	0.25	128	>128	128	64	25		1	0.25	128	>128	128	128	>50
	0.0156	0.0156	>128	>128	>128	>128	6.25		0.0156	0.0156	4	>128	>128	>128	6.25
AS-1664 	0.0078	0.0078	>128	>128	>128	>128	12.5	AS-1934 	0.0312	0.0625	>128	>128	>128	>128	>50
	0.0156	0.0078	>128	>128	128	128	6.25		2	2	4	>128	>128	>128	>50
	2	1	128	>128	>128	128	12.5		1	0.5	>128	>128	128	64	>50
	1	0.5	>128	>128	>128	64	12.5	Clarithromycin	0.0078	0.0078	<0.125	0.5	16	8	NT
	0.0078	0.0156	>128	>128	>128	>128	1.56	Ampicillin	0.25	0.125	<0.125	0.5	4	0.5	NT
	0.25	0.5	>128	>128	>128	>128	6.25								

Minimum Inhibitory Concentration (MIC・μg/ml)

1 16

Maximum tolerated dose (MTD; mg/kg)

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Anti-*H. pylori* Activities of Intervenolin derivatives

H. pylori

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

MIC（μg/ml） 1 16

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

Anti-Microbial Activities of Intervenolin derivatives

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

MIC（μg/ml）
1 16

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

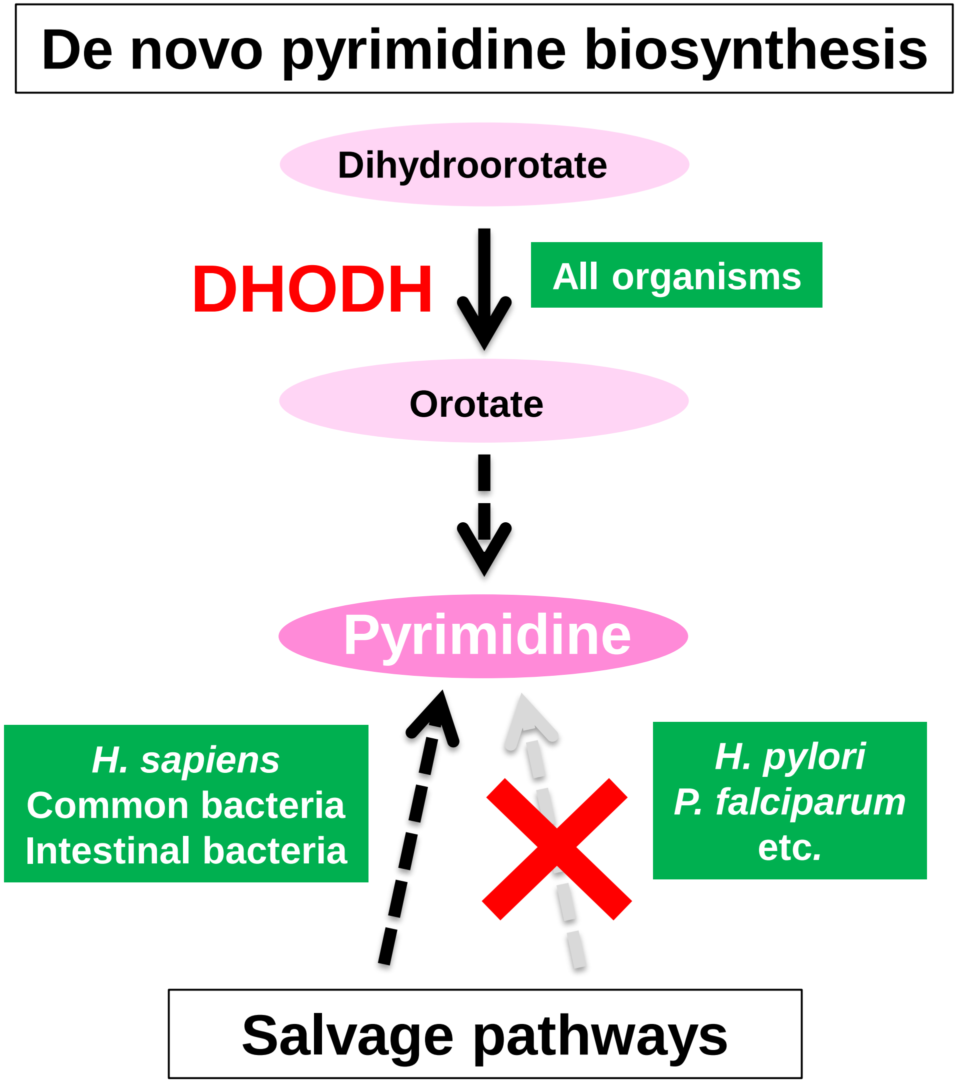
Anti-Intestinal Bacterial Activities of Intervenolin derivatives

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

MIC (µg/ml)
1 16

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

Biosynthetic Pathway of Pyrimidine

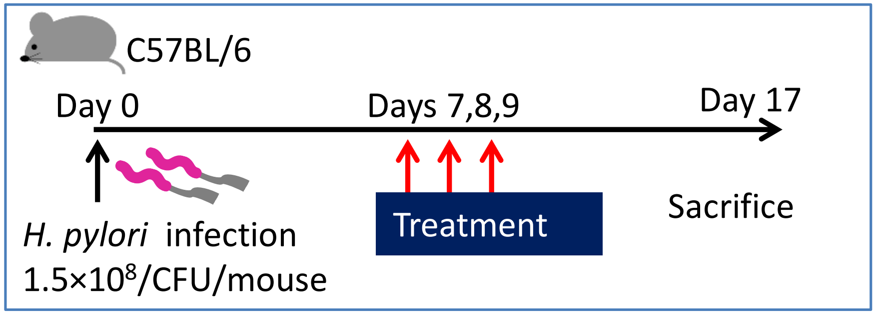


Triple Therapy of *H. pylori*

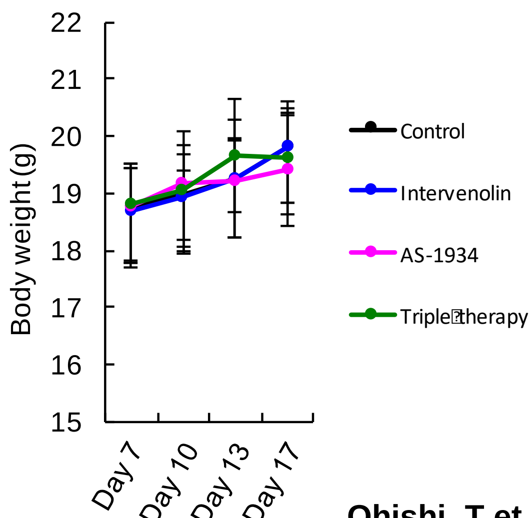
- Proton pump inhibitor (PPI)（Omeprazole）20 mg/shot
- Penicillin antibiotic： Amoxicillin 750 mg/shot
- Macrolide antibiotic： Clarithromycin　200 mg/shot

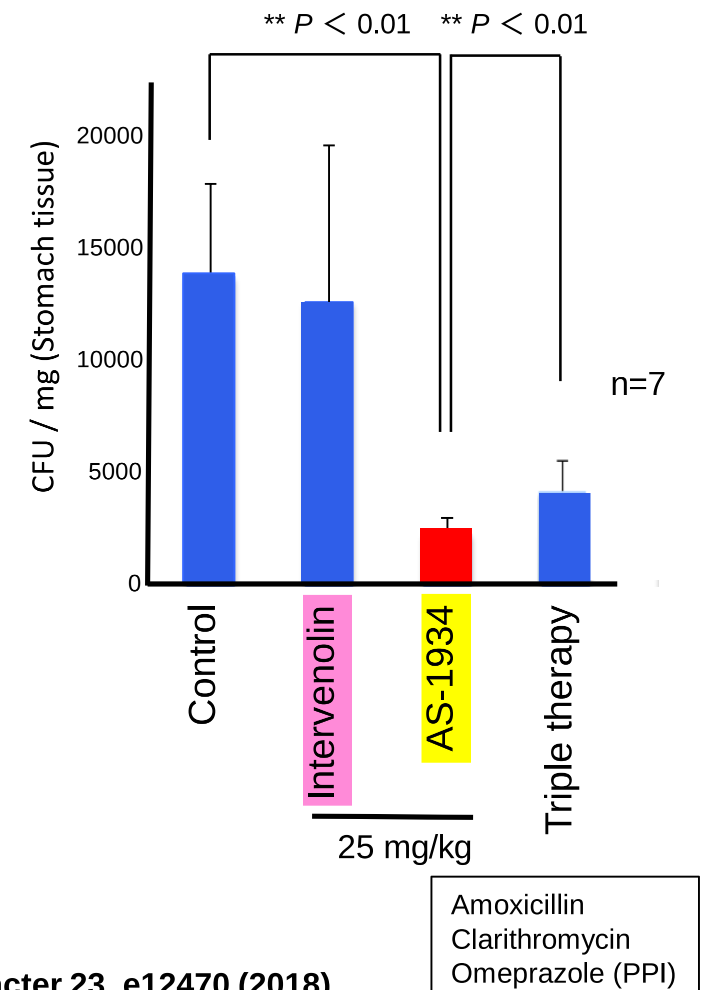
Twice a day for 7 days

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



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

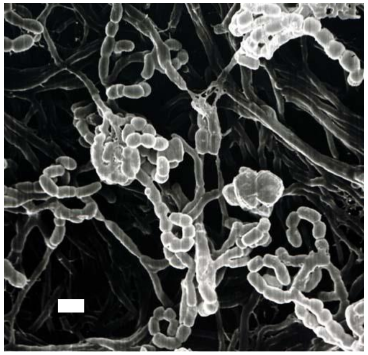




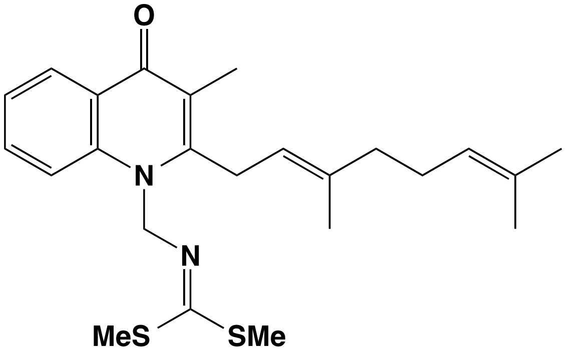
Ohishi, T et al, Helicobacter 23, e12470 (2018)

Intervenolin, a New Compound, with Anti-tumor and Anti-*H. pylori* Activities

Nocardia **sp. ML96-86F2**



***Bar*=1 μm**



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P-CREATE
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