

# Chemistry and Biology of Azoprenylated Secondary Metabolites

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## Prenylation in nature

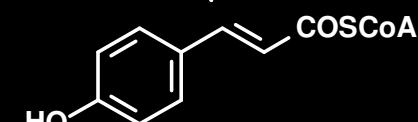
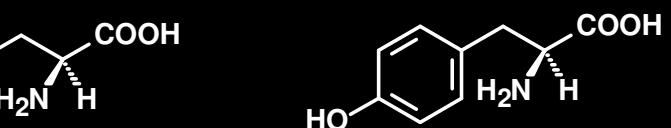
Aminoacids

1-DOXP

Mevalonate

Alkaloids

Terpenes



Phenylpropanoids

Prenylazoalkaloids

Prenyloxyphenylpropanoids

Prenyloxyalkaloids

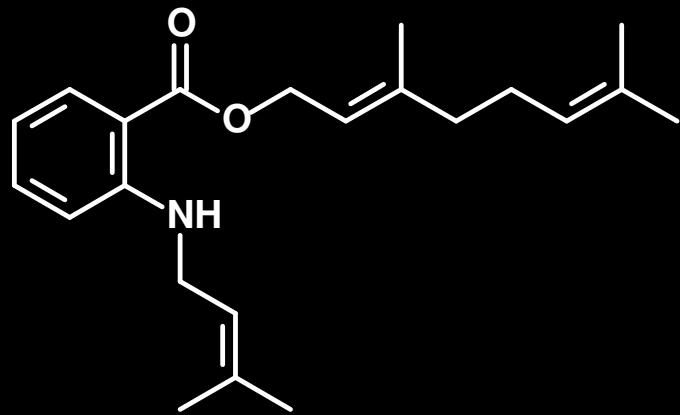


## Azoprenylated Secondary Metabolites

- ANTHRANILIC ACID DERIVATIVES
- DIAZEPINONES
- INDOLE ALKALOIDS
- XANTHINE ALKALOIDS



## Anthranilic acid derivatives

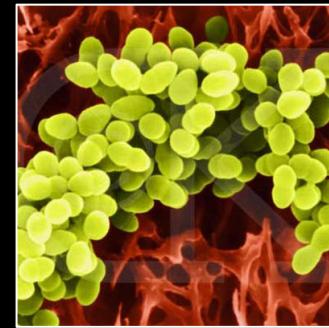
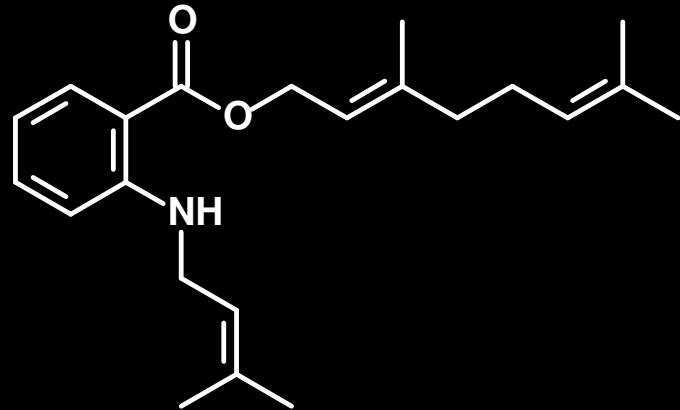


***Esenbeckia berlandieri* Baillon ex Hemsley  
(Rutaceae)**

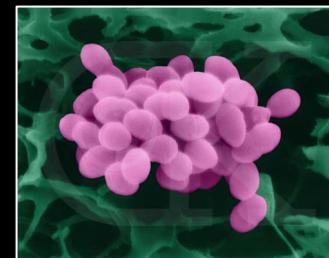
Aguilar-Guadarrama et al. *Planta Med*, 2004, 70



## Anti-microbial activity



***Staphylococcus aureus* (ATCC 29213)**  
**MIC = 200 µg/mL**



***Staphylococcus faecalis***  
**INACTIVE**



## Diazepinones



**Diazepinomicin**



***Micronospora* spp.**

Charan et al. *J. Nat. Prod.*, 2004, 167

McAlpine et al., 2008, 1585



## Diazepinomicin



**Peripheral BZD receptor**

$IC_{50} = 0.291 \mu M$

**GABA<sub>A</sub> receptor = no affinity**



## Diazepinomicin: *in vitro* anti-cancer activity

Cancer type	GIC <sub>50</sub> range ( $\mu$ M)
Leukemia	1.1–1.8
Non-small cell lung carcinoma	0.23–13.6
Melanoma	1.3–46.7
Prostate carcinoma	3.9–12.9
Breast carcinoma	0.11–18.3
Ovarian carcinoma	0.71–30.7
Renal carcinoma	1.5–22.3
Colon carcinoma	1.3–20.7
Malignant CNS glioma	1.4–13.0



## Diazepinomicin: activity on overexpressed BZD receptor tumors

Tumor type	Origin	Cell line	IC <sub>50</sub> (μM)*
Oligodendrogloma	Human	GHA	1.6 ± 0.7
		DN	3.0 ± 0.7
Astrocytoma	Human	U373	3.8 ± 1.4
		GHD	6.5 ± 2.9
Glioblastoma	Mouse	GL26	8.9 ± 1.1
	Human	U-87MG	10.9 ± 0.5
		SNB-19	5.3 ± 1.3
Gliosarcoma	Rat	C6	4.3 ± 2.3
	Rat	9L	8.3 ± 3.8

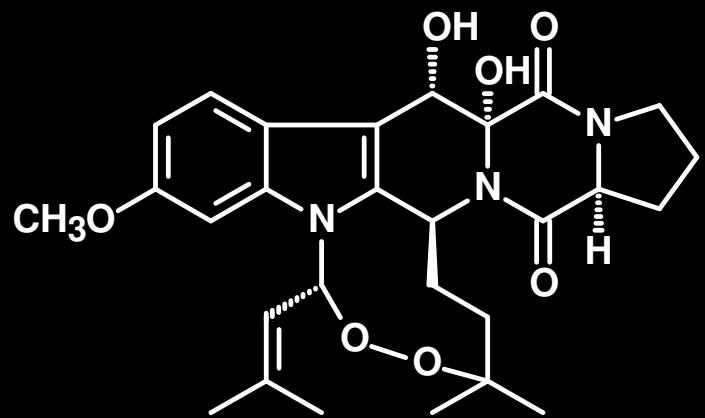


## Diazepinomicin: *in vivo* anti-cancer activity

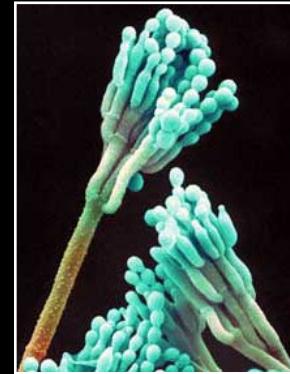
Tumor	Administration route	Dose (mg/kg)	Inhibition (%)
Rat C6 glioma	Intraperitoneal	20 (1st week)	66
		10 (2nd week)	
	Subcutaneous	30 (1st week)	60
		15 (2nd week)	
U87MG	Subcutaneous	30	36
MDA-MB-231	Subcutaneous	30	72
		20	66
	Intraperitoneal	30	56
PC3	Subcutaneous	30	85-97



## Indole alkaloids



**Verruculogen**



***Penicillium* spp.**

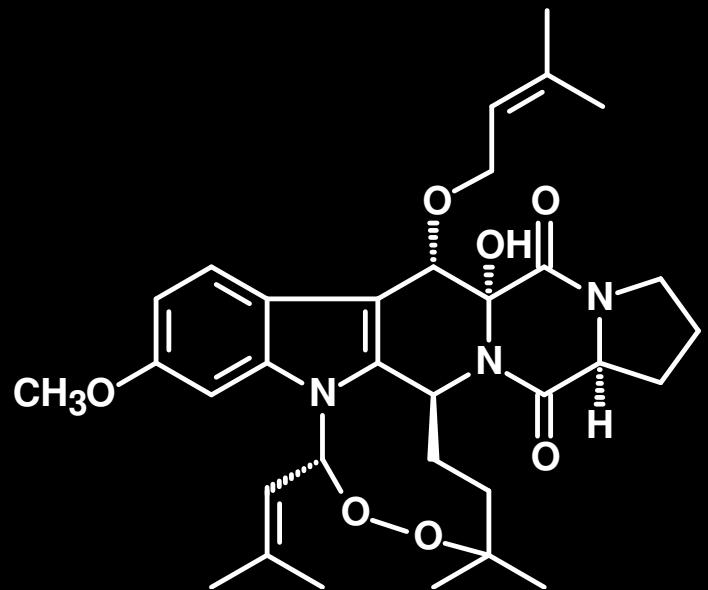


***Aspergillus fumigatus***

Kosalec et al., *Acta Pharm.* 2005, 357 and refs. cited herein



## Indole alkaloids



Fumitremorgin A

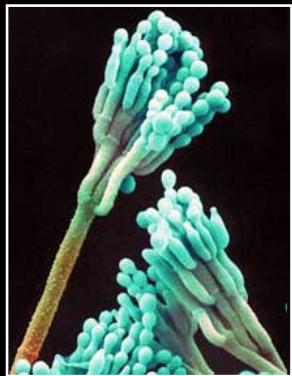


Fumitremorgin B

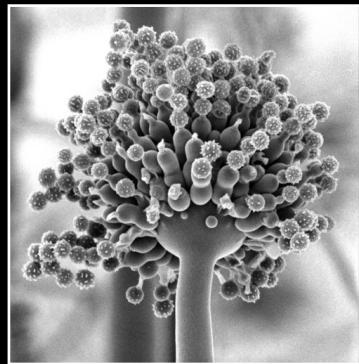
Dos Santos et al., *Mycopathologia*. 2003, 133 and  
refs. cited herein



## Fumitremorgins



***Penicillium* spp.**



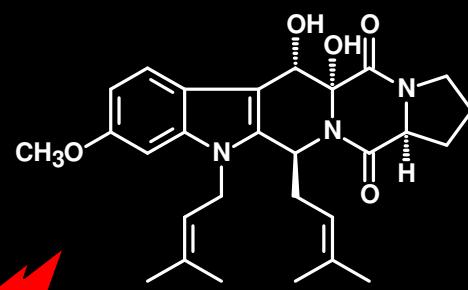
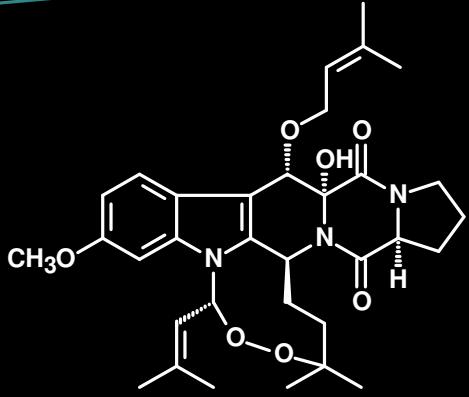
***Aspergillus* spp.**



***Neosartorya fischeri***

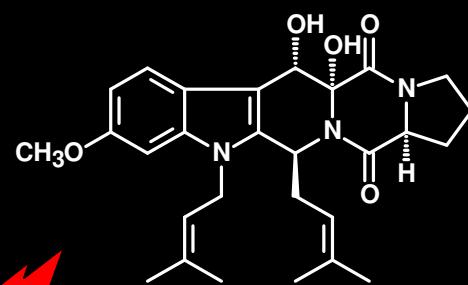
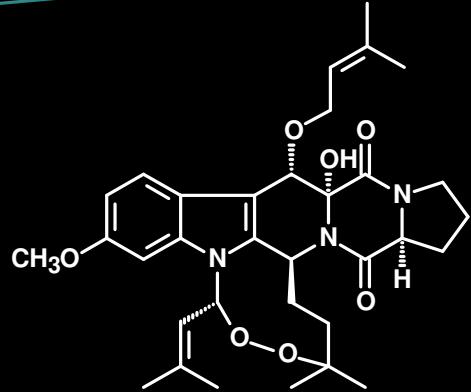


## Verruculogen and Fumitremorgins





## Verruculogen and Fumitremorgins





## Verruculogen: anti-proliferative activity



**ftTS210 cells**  
**Block in the M phase**  
**MIC = 12.2  $\mu$ M**



## Indole alkaloids



**13-Oxofumitremorgin B**

*Sordaria gondaensis*



*Stichopus japonicus*

Wang et al., *Tetrahedron* 2008, 7986



## Indole alkaloids

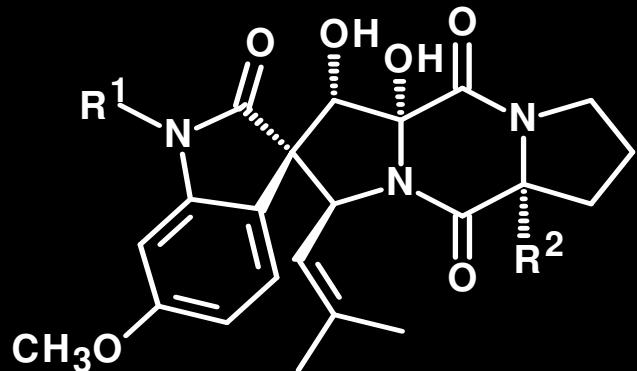


***N*-prenyl-cyclo-L-tryptophyl-L-proline**

Wang et al., *Tetrahedron* 2008, 7986



## Indole alkaloids



R<sup>1</sup> = isopentenyl; R<sup>2</sup> = H

R<sup>1</sup> = isopentenyl; R<sup>2</sup> = OH



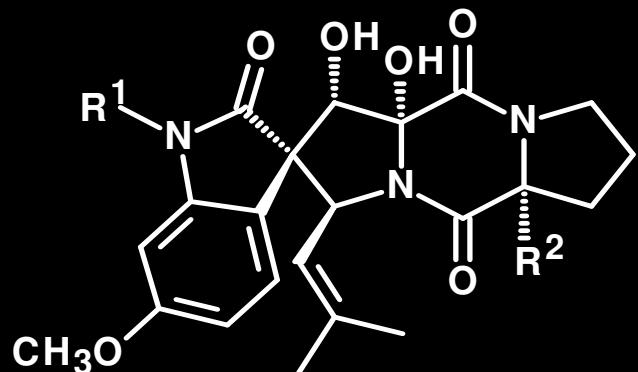
### Spirotryptostatins A-C



*Stichopus japonicus*

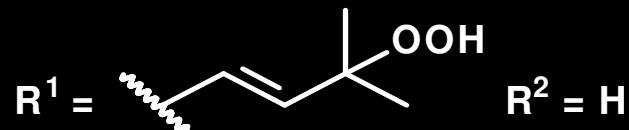


## Spirotryptostatins: *in vitro* anti-proliferative activity



$R^1$  = isopentenyl;  $R^2$  = H

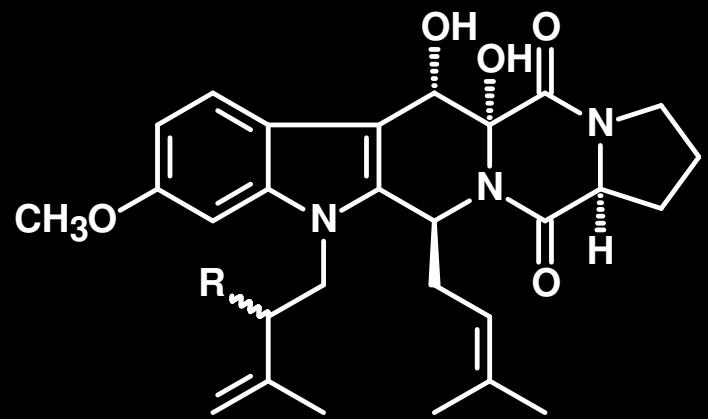
$R^1$  = isopentenyl;  $R^2$  = OH



Cytotoxicity ( $IC_{50}$ , $\mu M$ )			
MTT		SRB	
MOLT-4	HL-60	A-549	BEL-7402
25.7	43.5	35.9	68.8
25.7	45.0	35.5	17.5
3.1	2.3	3.1	98.4



## Indole alkaloids

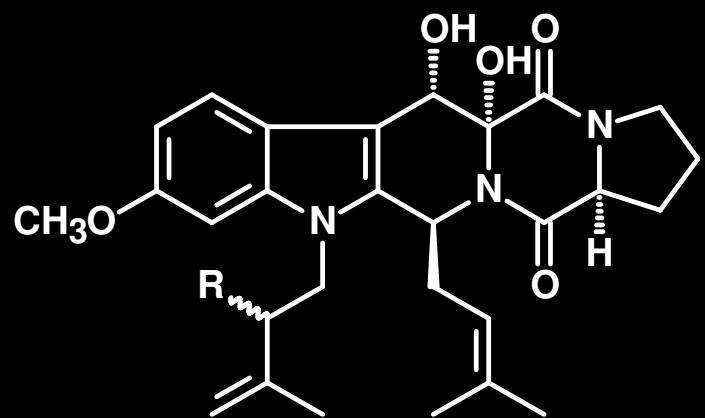


$\text{R} = \alpha\text{-OH}$

$\text{R} = \beta\text{-OH}$



## *In vitro* anti-proliferative activity



$\text{R} = \alpha\text{-OH}$

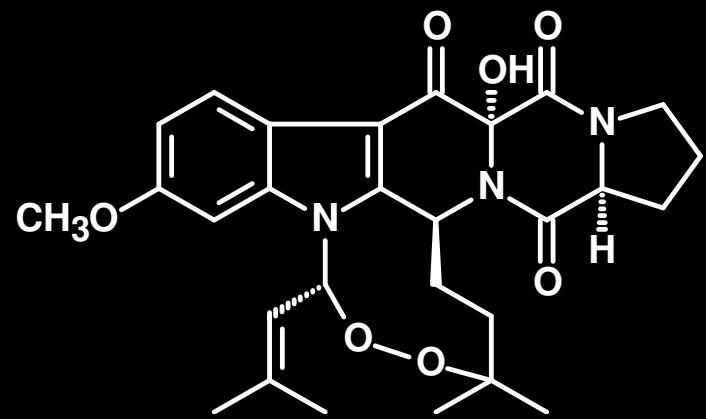
$\text{R} = \beta\text{-OH}$

Cytotoxicity ( $\text{IC}_{50}$ ,  $\mu\text{M}$ )

MTT	SRB		
MOLT-4	HL-60	A-549	BEL-7402
11.0	3.4	11.0	7.0
11.0	5.4	11.6	10.8



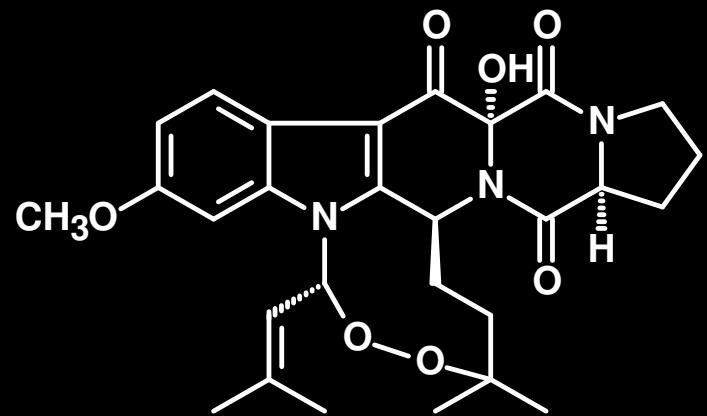
## Indole alkaloids



**13-Oxoverruculogen**



## 13-Oxoverruculogen: *in vitro* anti-proliferative activity

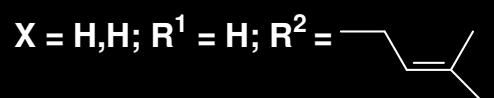
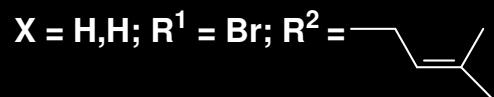
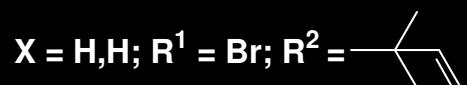
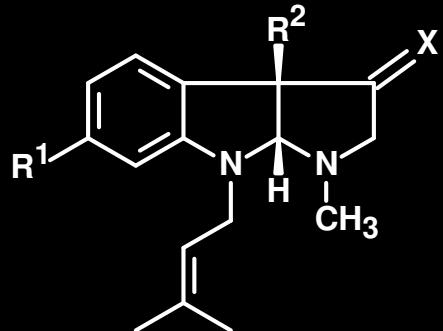


Cytotoxicity ( $IC_{50}$ ,  $\mu M$ )

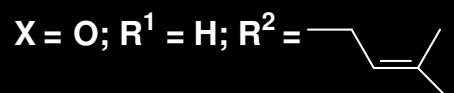
MTT	SRB		
MOLT-4	HL-60	A-549	BEL-7402
25.7	1.9	16.9	25.6



## Flustramines and flustramides



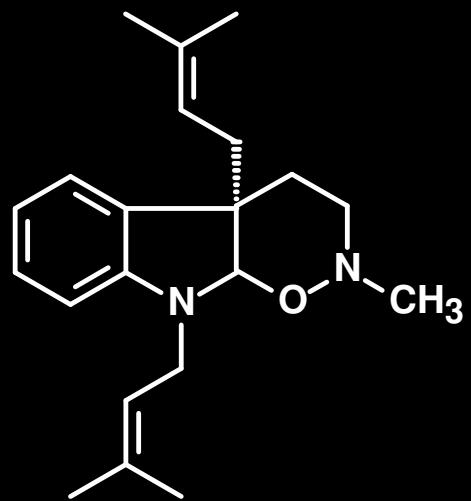
*Flustra foliacea* L.



Carle et al., *J. Org. Chem.* 1980, 1586



## Flustramines and flustramides

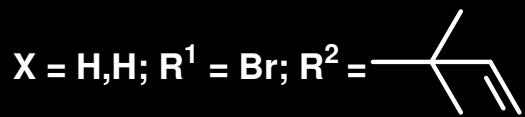
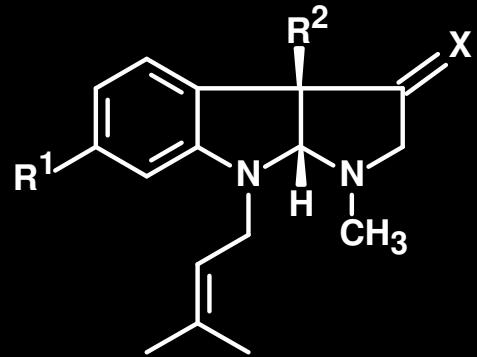


**Flustramine *N*-oxide**

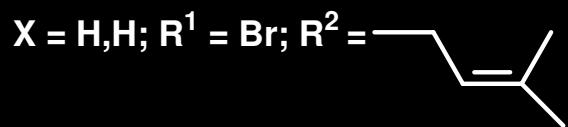




## Flustramines and flustramides



**Flustramine A**



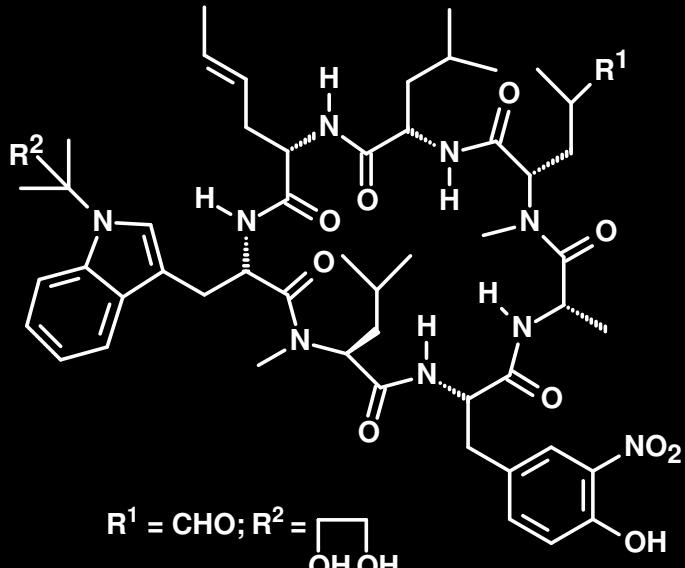
**Flustramine B**

**Muscle relaxation**

**(phenylephrine pre-contracted  
aorta guinea pig)**



## Indole "reverse" N-alkaloids



**Rufomycin A**

$R^1 = \text{CH}_3; R^2 = \text{CH}=\text{CH}_2$

**Rufomycin B**

$R^1 = \text{CHO}; R^2 = \begin{array}{c} \text{O} \\ | \\ \text{C} \\ | \\ \text{O} \end{array}$

**Illamycin A**

$R^1 = \text{CH}_3; R^2 = \text{CH}=\text{CH}_2$

**Illamycin B**

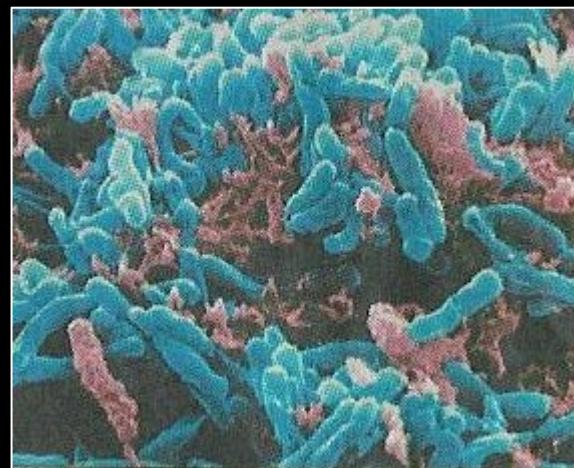
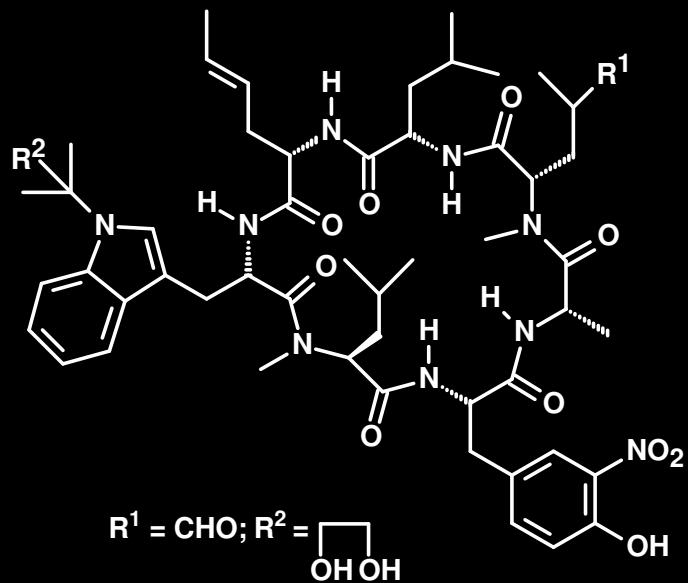


***Streptomyces atratus***

**Shibata et al., Agr. Biol. Chem. 1962, 228**



## Indole "reverse" N-alkaloids



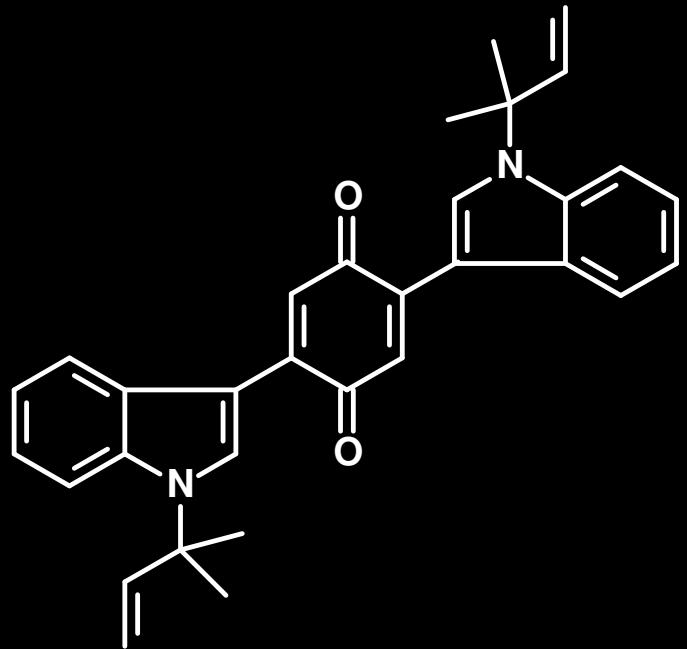
**Anti-bacterial activity  
(*Mycobacterium* spp.)**

**MIC = 0.1 – 5.0  $\mu\text{g/mL}$**

$R^1 = \text{CH}_3; R^2 = \text{CH}=\text{CH}_2$



## Asterriquinone

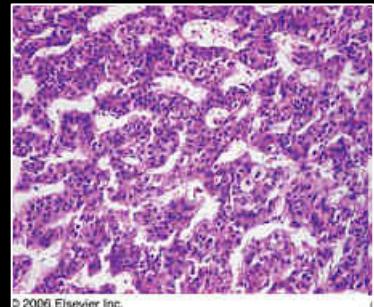
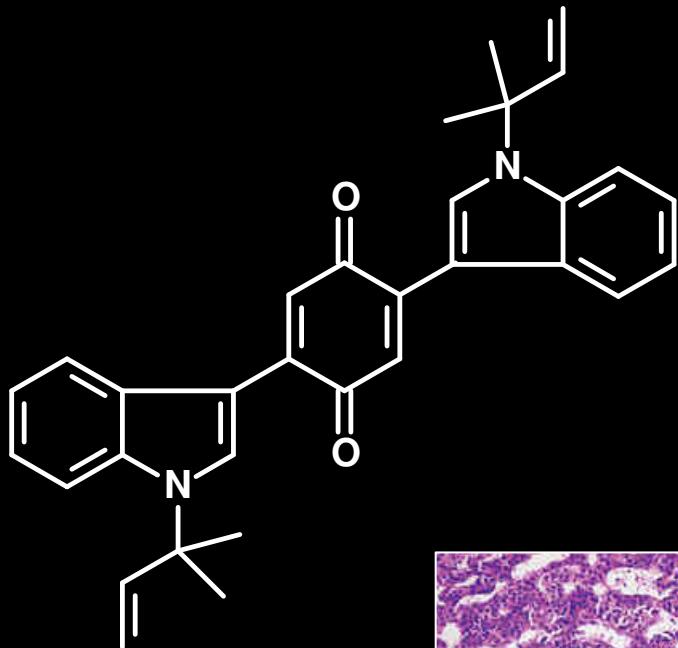


*Aspergillus terreus*

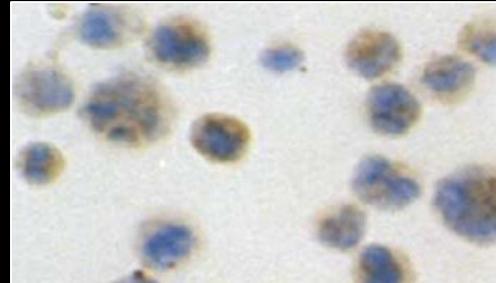
Yamamoto et al., *Chem. Pharm. Bull.* 1976, 1853



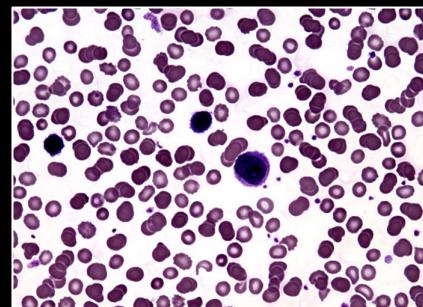
## Asterriquinone: *in vivo* anti-cancer activity



### Ehrlich ascites carcinoma



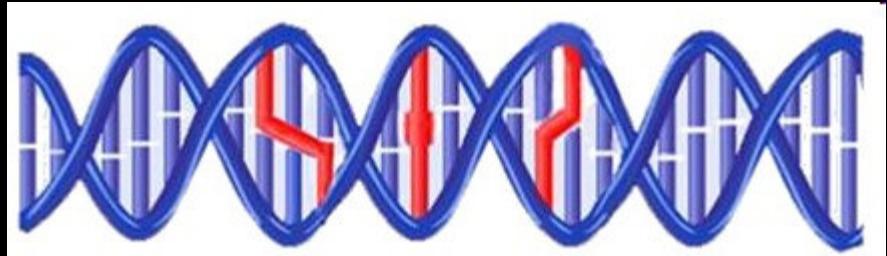
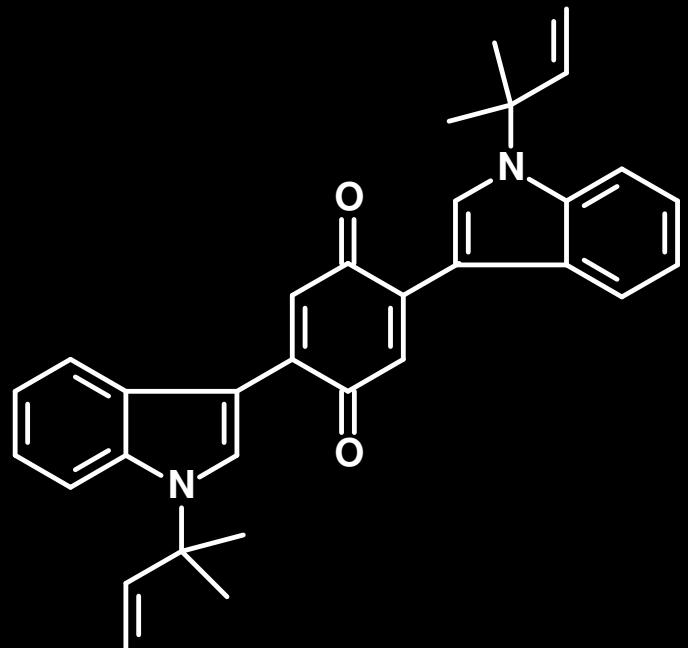
### Lymphatic leukemia L1210



### Ascites hepatoma AH109A



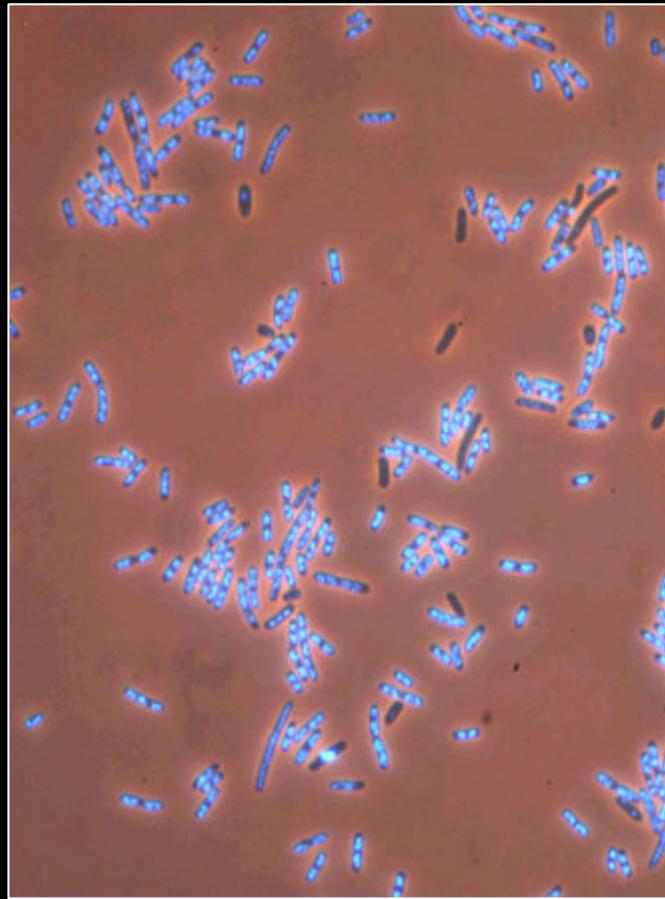
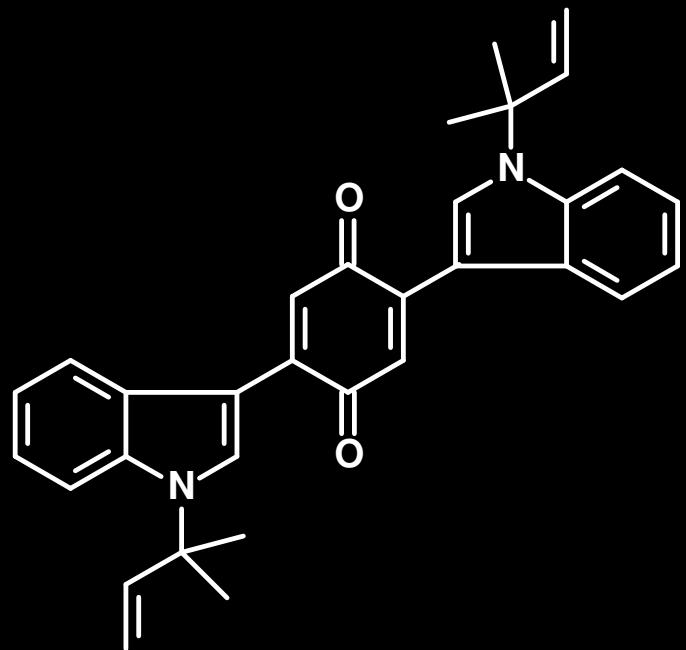
## Asterriquinone: interaction with DNA



Kaji et al., *Anticancer Res.* 1997, 3675

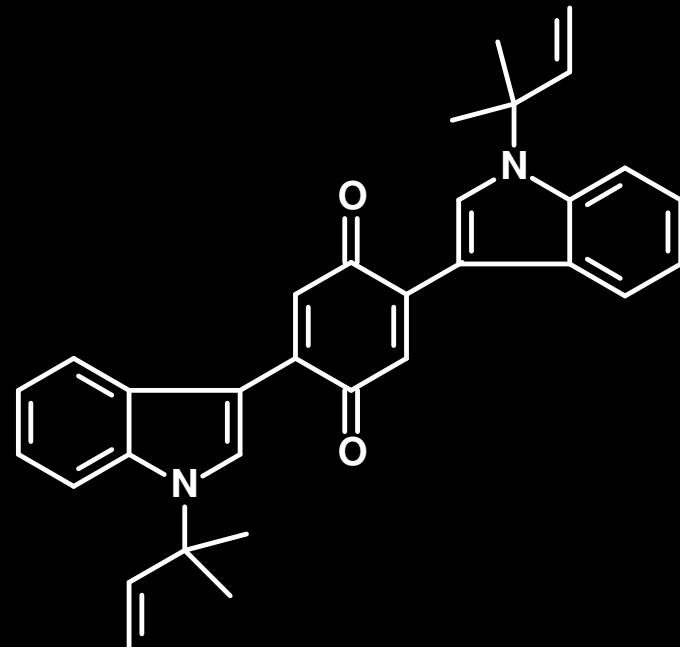
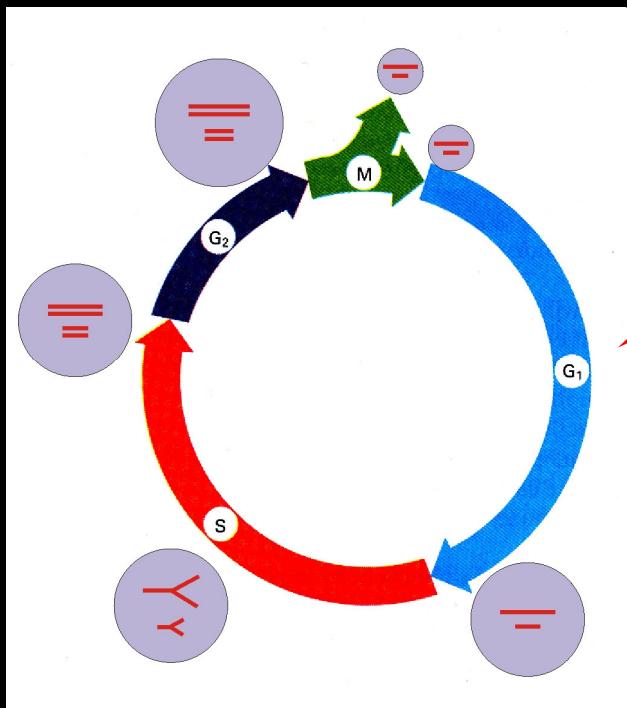


## Asterriquinone: interaction with DNA



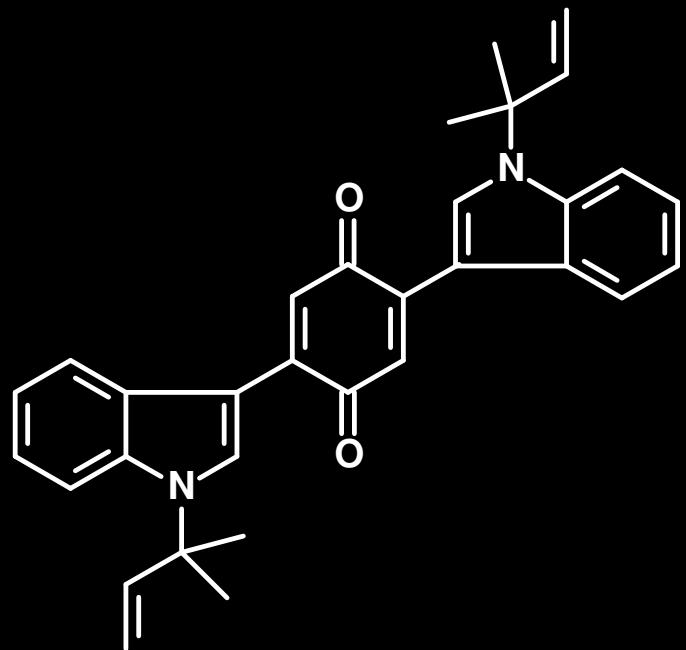


## Asterriquinone: interaction with DNA





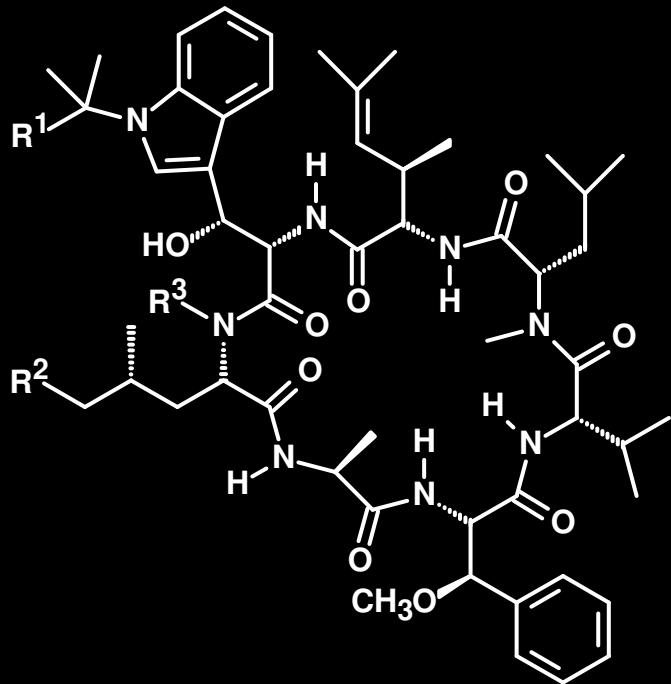
## Asterriquinone: *in vitro* anti-HIV activity



**HIV-1 reverse transcriptase**  
**10  $\mu$ M = 70% inhibition**



## Cyclomarins A-D

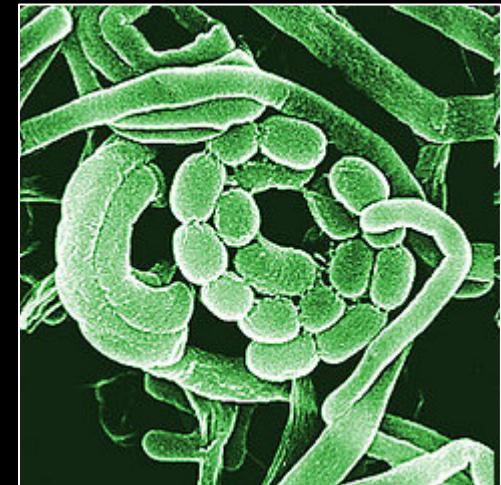


$\text{R}^1 = \text{CH}=\text{CH}_2; \text{R}^2 = \text{OH}; \text{R}^3 = \text{CH}_3$

$\text{R}^1 = \text{CH}=\text{CH}_2; \text{R}^2 = \text{H}; \text{R}^3 = \text{CH}_3$

$\text{R}^1 = \text{CH}=\text{CH}_2; \text{R}^2 = \text{OH}; \text{R}^3 = \text{CH}_3$

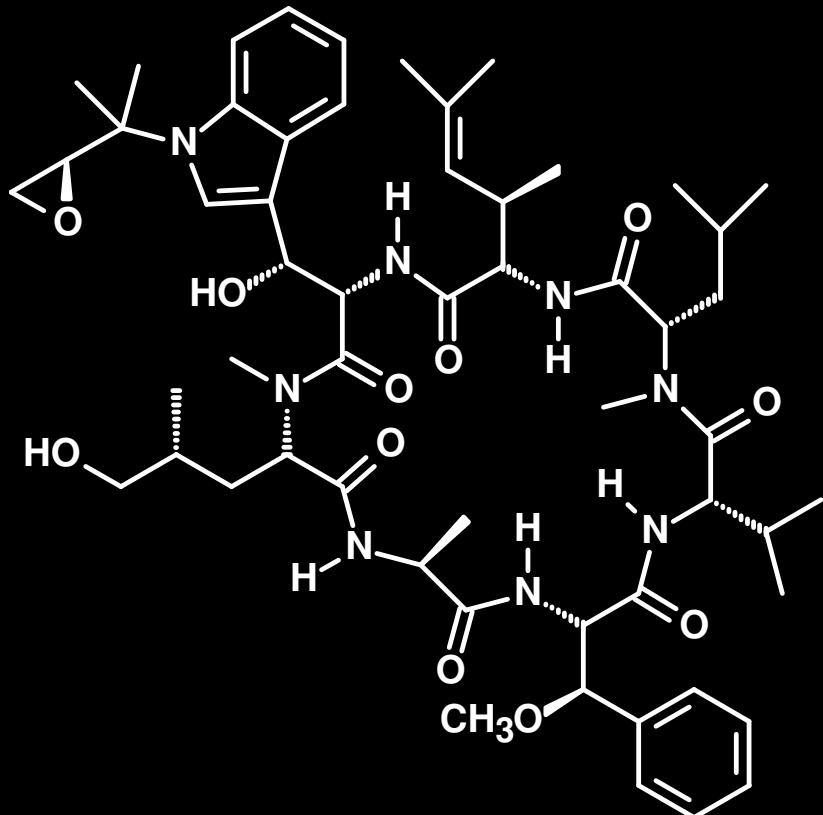
28:  $\text{R}^1 = \text{CH}=\text{CH}_2; \text{R}^2 = \text{OH}; \text{R}^3 = \text{H}$



***Streptomyces*  
strain CN-982**



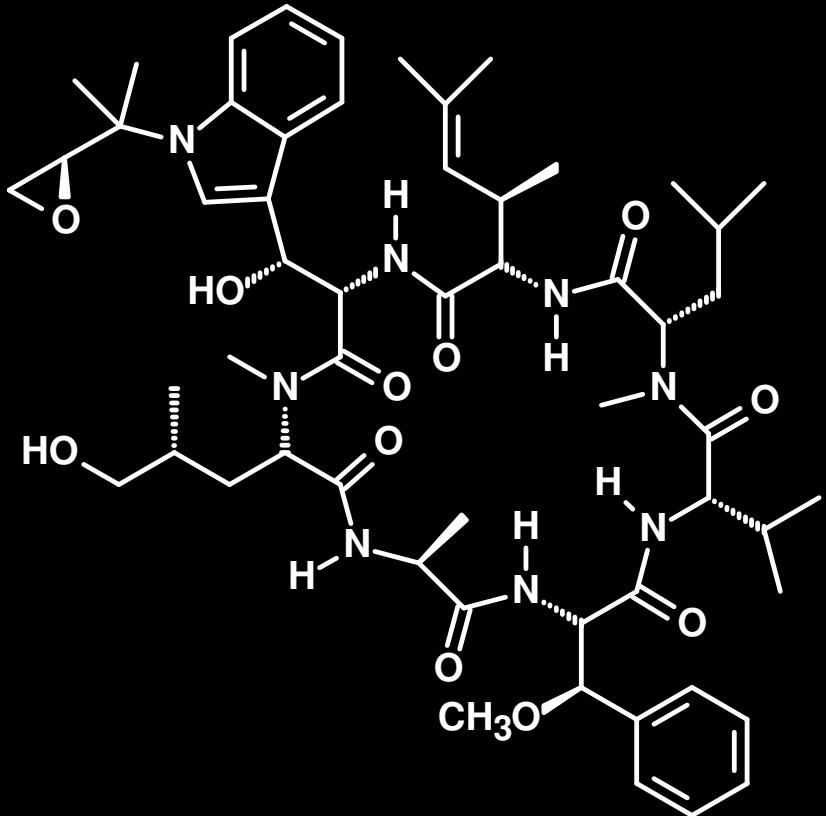
## Cyclomarin A: anti-cancer activity



**Mean IC<sub>50</sub> = 2.6 μM**



## Cyclomarin A: anti-inflammatory activity



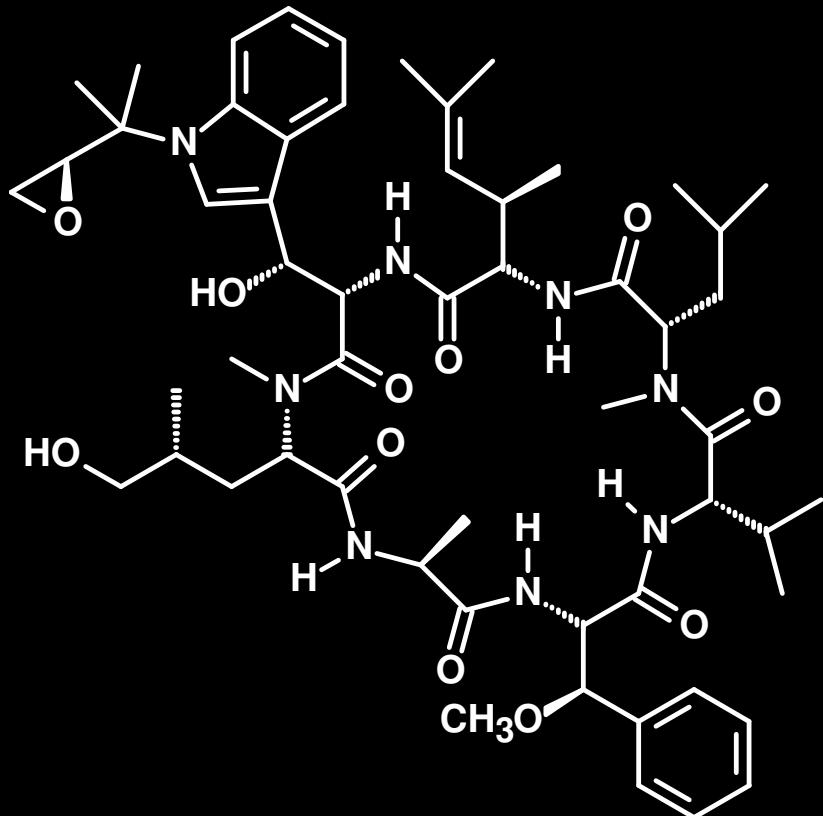
Phorbol esters induced ear oedema in mice (50 µg/ear)

**96% inhibition**

**Indomethacin 72% inhibition**



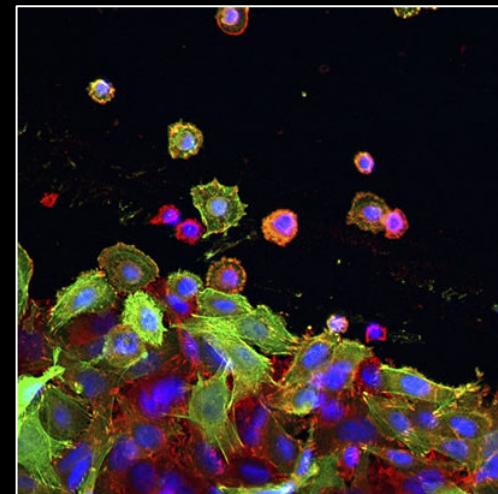
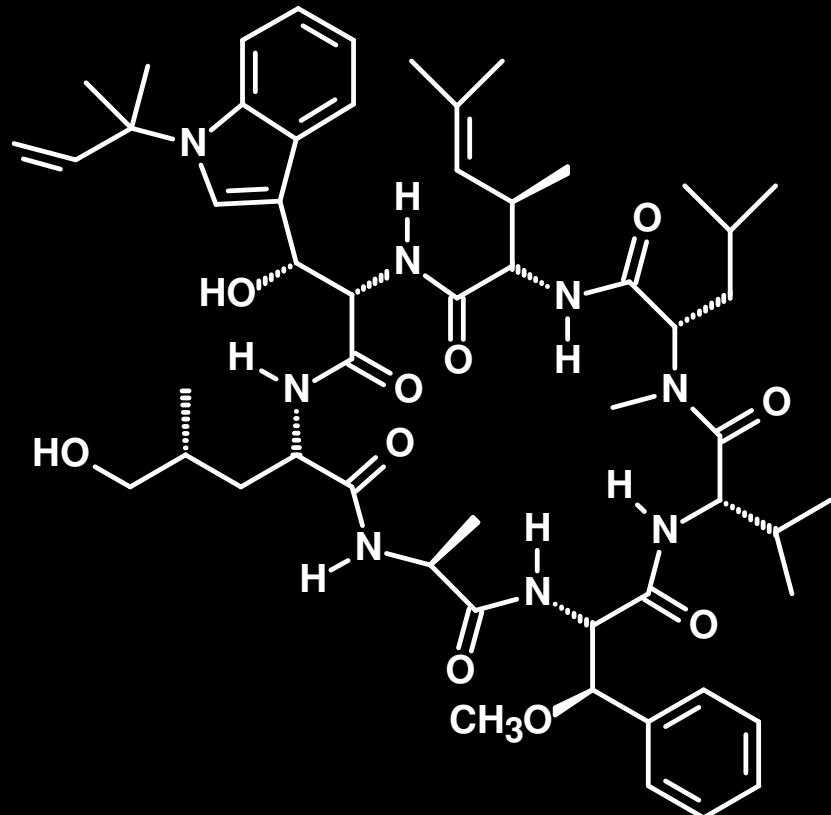
## Cyclomarin A: anti-viral activity



**Marinovir® - Phytera Inc.**



## Cyclomarine D: *in vitro* anti-cancer activity

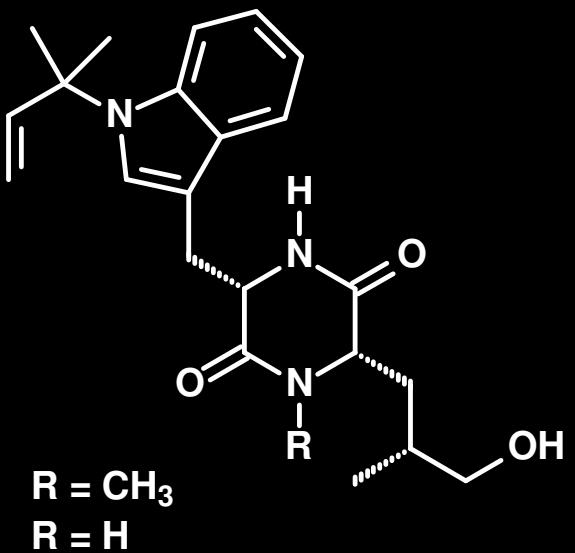


**Colon cancer cells HT-116**

**IC<sub>50</sub> = 2.0 µg/mL**



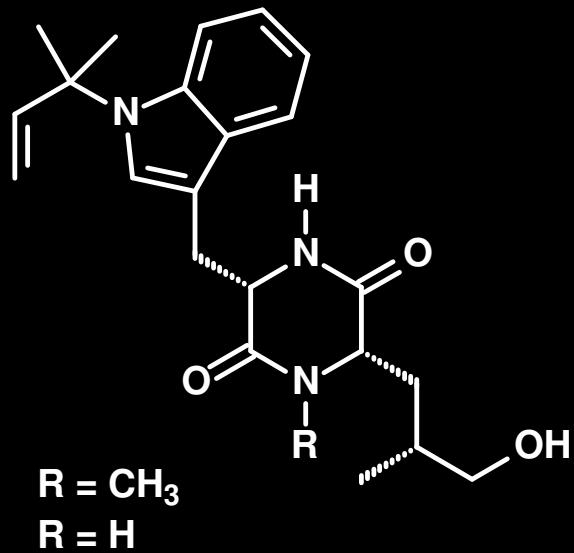
## Cyclomarazines A and B



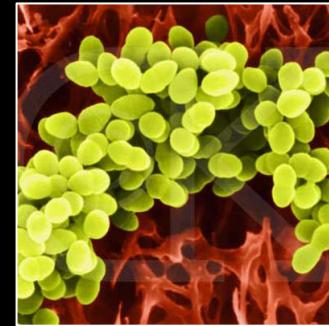
***Salinispura aericola***



## Cyclomarazines A and B: anti-bacterial activity



**MIC = 13 – 18 µg/mL**



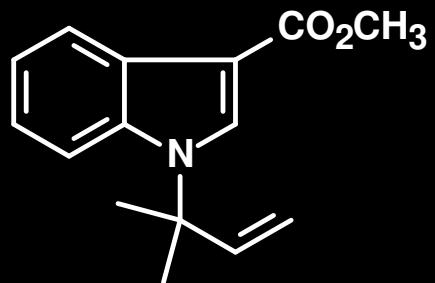
***Staphylococcus aureus***



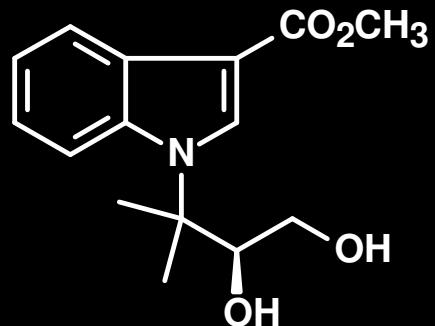
***Enterococcus faecium***



## Indole "reverse" N-alkaloids

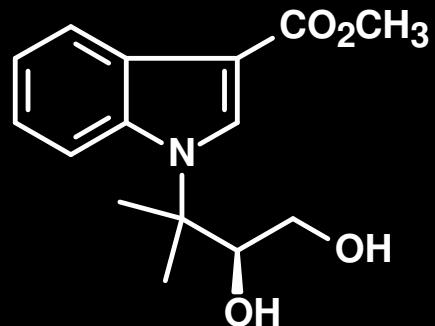
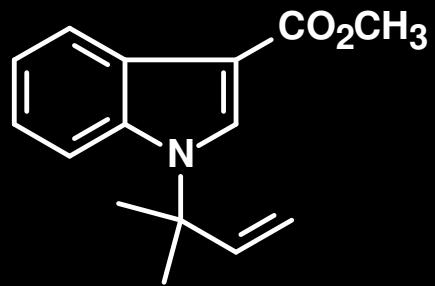


**Aporpium caryae**





## Indole "reverse" N-alkaloids



**Mild *in vitro* antifungal activity  
(*Cardiosporum cucumerinum*)**



## Xanthine alkaloids



***Bosistoa floydii* T.G. Hartley  
(Rutaceae)**



## Conclusions and future perspectives

- ❖ **N-PRENYL SECONDARY METABOLITES ARE MAINLY FOUND IN FUNGI, BACTERIA, AND MARINE ORGANISMS**
- ❖ **N-PRENYL SECONDARY METABOLITES ARE EXTRACTED FROM NATURAL SOURCES IN VERY LOW CONCENTRATIONS**
- ❖ **LACK OF PHARMACOLOGICAL STUDIES**
- ❖ **N-PRENYL SECONDARY METABOLITES COULD BE SYNTHESIZED IN GOOD YIELD**
- ❖ **HANDLING OF QUANTITIES THAT ARE SUFFICIENT TO PERFORM *IN VITRO* AND *IN VIVO* PHARMACOLOGICAL TESTS**



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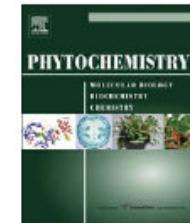


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Review

### Chemistry and biological activity of azoprenylated secondary metabolites

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