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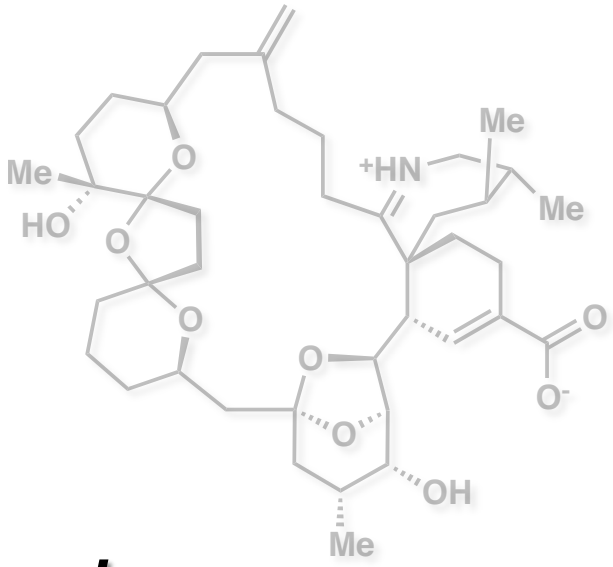
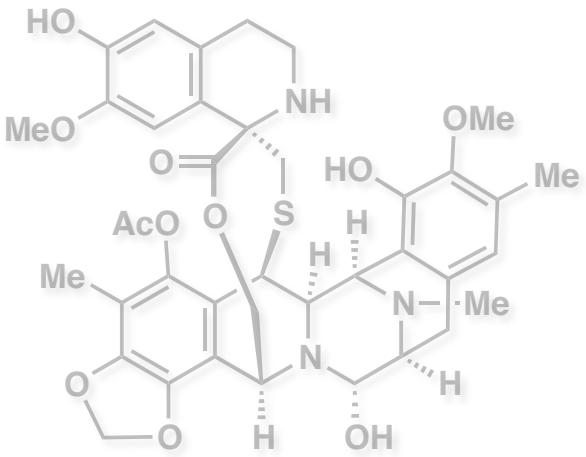


Iminium Ion Pharmacophores



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Shenvi Group

Iminium Ion Pharmacophores

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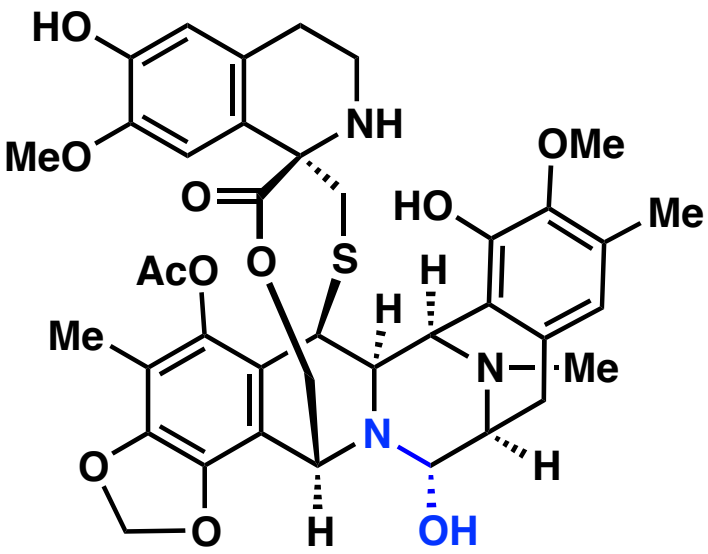
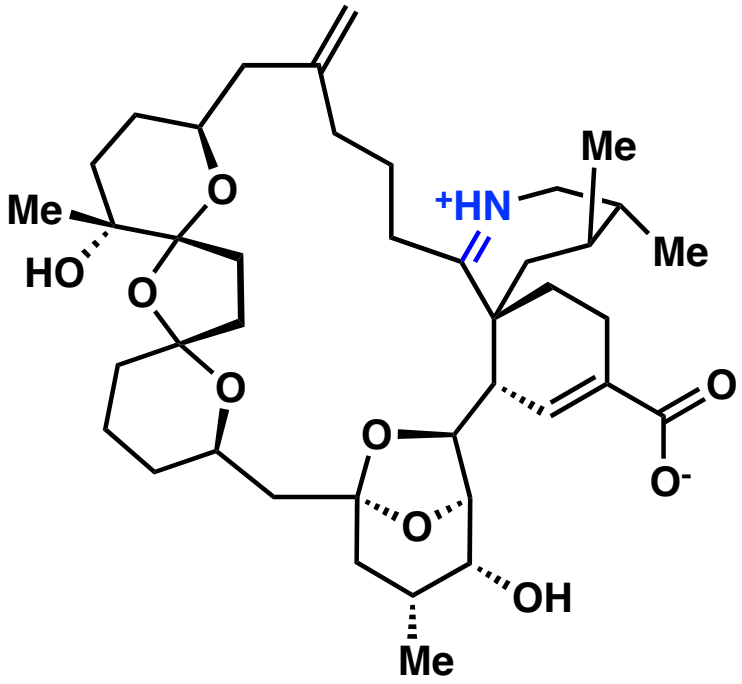
Shenvi Group

Tetrahydroquinoline Natural Products



Cyclic Imine Phycotoxines



Tetrahydroisoquinoline Natural Products

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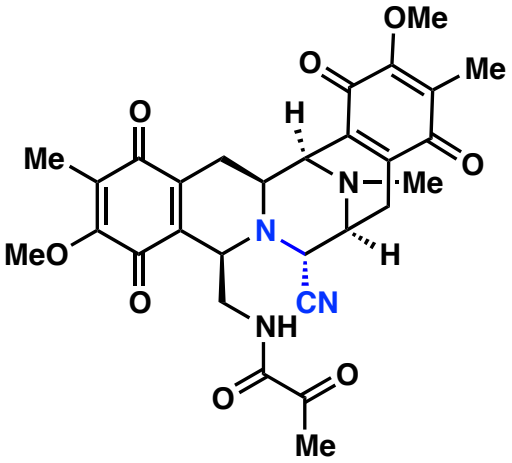
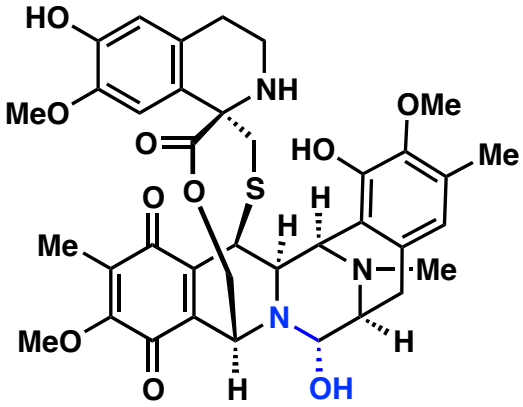
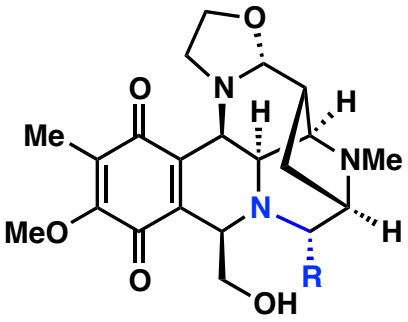


Saframycin A

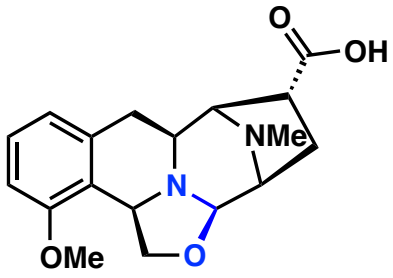
Over 50 members to date



Ecteinascidin 743



Naphthyridinomycin (R = OH)
Cyanocycline A (R = CN)



Quionocarcin

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Saframycins

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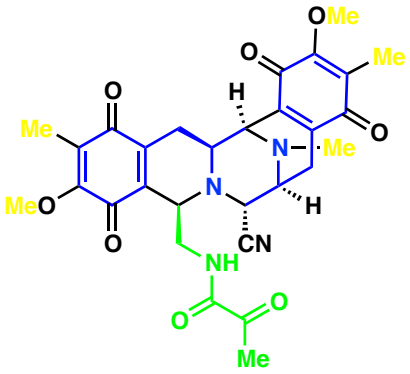


Saframycin A

Isolated in 1977 by Arai and co-workers from *Streptomyces lavendulae*
Some 18 members are know to date

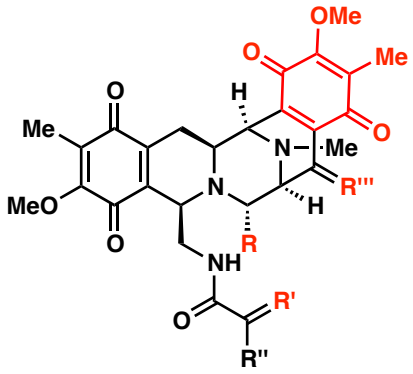
Structure determined in 1980s by NMR and X-ray (saframycin C)

Biosynthesis involves NRPS system, heme peroxidase, and *O*-Me-transferase
Two tyrosins, one glycin, one alanine, and five methionines are utilized

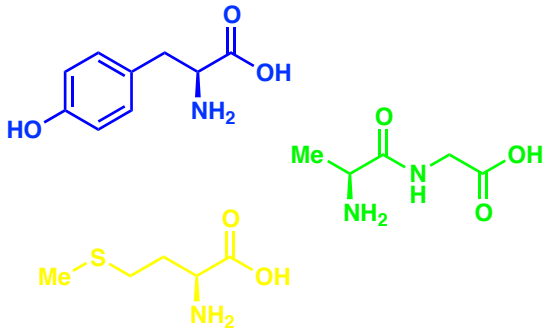
Potent antimicrobial activity (MICs as low as 0.05 μg/mL)
Potent antiproliferative activity (ID_{50} = 5.3, 5.6 nM for A375 and L1210)

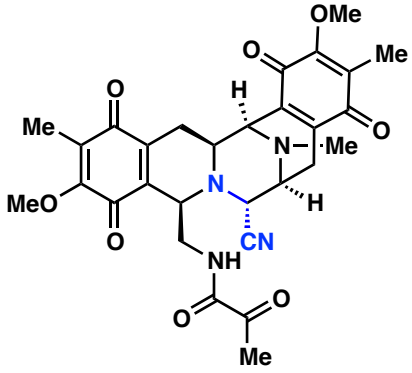
Potent antitumor activity (90% survival at 0.5 mg/kg/day after 40 days
vs 0% survival in control mice after 18 days,
Ehrlich ascites tumors, saframycin S)

GAPDH was demonstrated to be a cellular target (Myers, 2004)



Saframycins

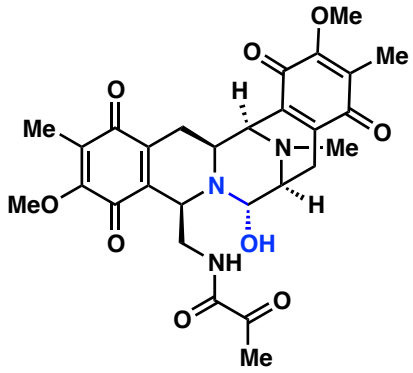




Saframycin A

$0.1\ N\ H_2SO_4$

NaCN



Saframycin S

Saframycins

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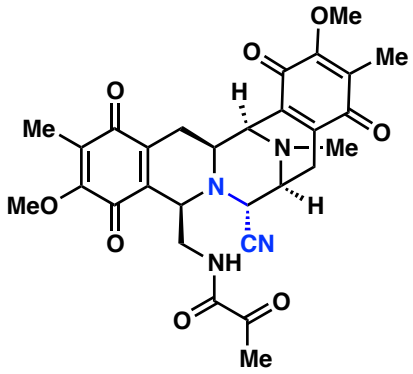
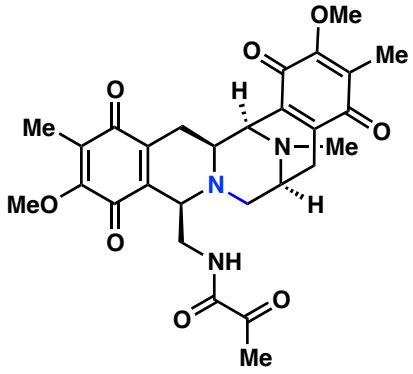
Table 1. Antimicrobial spectra of saframycins

Test organism	MIC (mcg/ml)				
	A	B	C	D	E
Staphylococcus aureus FDA 209P	0.1	12.5	25.0	25.0	100.0
Staphylococcus aureus Smith	0.05	1.56	6.25	50.0	50.0
Staphylococcus albus	0.1	12.5	25.0	50.0	100.0
Staphylococcus citreus	0.1	12.5	25.0	50.0	100.0
Streptococcus faecalis	6.25	>100.0	>100.0		>100.0
Streptococcus pyogenes Coock*	0.78	12.5	25.0		>100.0
Streptococcus pyogenes 090R*	6.25	25.0	12.5		25.0
*Streptococcus salivarius**	6.25	100.0	>100.0		>100.0
Sarcina lutea	0.05	1.56	6.25	50.0	12.5
Bacillus subtilis PCI 219	0.1	25.0	25.0	50.0	100.0
Bacillus cereus	12.5	100.0	100.0	100.0	25.0
Corynebacterium diphtheriae	<0.003	0.4	3.125	0.195	100.0
Corynebacterium xerosis	<0.003	12.5	25.0	6.25	25.0
Mycobacterium sp. 607	12.5	100.0	>100.0	50.0	100.0
Mycobacterium phlei	25.0	50.0	>100.0	50.0	25.0
Mycobacterium avium	12.5	100.0	>100.0	50.0	100.0
Nocardia asteroides	6.25	50.0	50.0	50.0	25.0
Escherichia coli F_1	50.0	>100.0	>100.0	>100.0	>100.0
Salmonella typhimurium	100.0	>100.0	>100.0	>100.0	>100.0
Shigella dysenteriae Shiga	25.0	>100.0	>100.0	>100.0	>100.0
Klebsiella pneumoniae	6.25	12.5	50.0	100.0	50.0
Brucella abortus	6.25	50.0	50.0	25.0	25.0
Serratia marcescens	>100.0	>100.0	>100.0	>100.0	>100.0
Pseudomonas aeruginosa	>100.0	>100.0	>100.0	>100.0	>100.0
Mucor mucedo	>100.0	>100.0	>100.0	>100.0	>100.0
Saccharomyces cerevisiae	>100.0	>100.0	>100.0	>100.0	>100.0
Rhodotorula glutinis	>100.0	>100.0	>100.0	>100.0	>100.0
Aspergillus niger	>100.0	>100.0	>100.0	>100.0	>100.0
Aspergillus oryzae	>100.0	>100.0	>100.0	>100.0	>100.0
Penicillium glaucum	50.0	>100.0	>100.0	>100.0	>100.0
Trichophyton mentagrophytes	>100.0	>100.0	>100.0	>100.0	>100.0
Candida albicans 7N	>100.0	>100.0	>100.0	>100.0	>100.0



Saframycin A



Saframycin B

Saframycins

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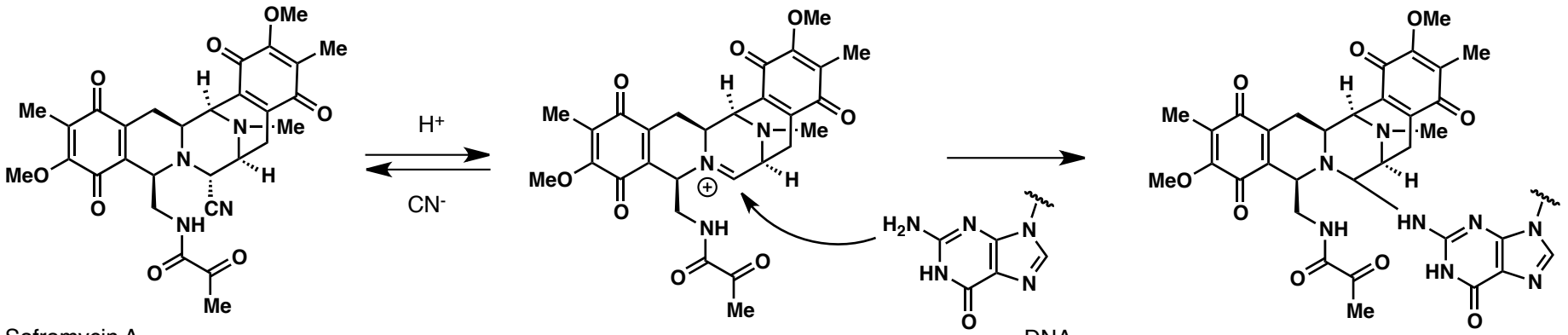
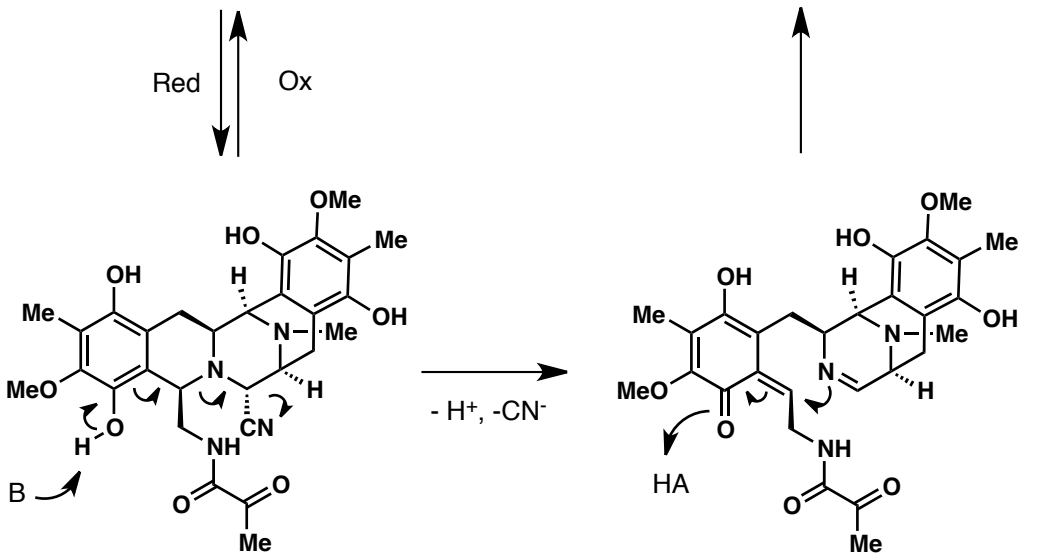
Lown and co-workers proposed first mechanism for alkylation of minor groove by saframycin A (1982)





DNA modification is believed to proceed via intial weak non-covalent binding followed by formation of mixed aminal with guanine residues

Participation of aminonitrile as a latent iminium electophile was supported by complete incorporation of ^{14}C-labeled tyrosine in DNA-saframycin conjugates and lack of ^{14}C incorporation by the natural products labeled at the CN-group

Higher potency of reduced saframycins and observation of DNA-cleavage suggests more than one mechanisms for cytotoxic effects of the natural products

Hill and Remers proposed alternative mechanism that accounts for increased antiproliferative activityies of reduced saframycins (1991)

Saframycins

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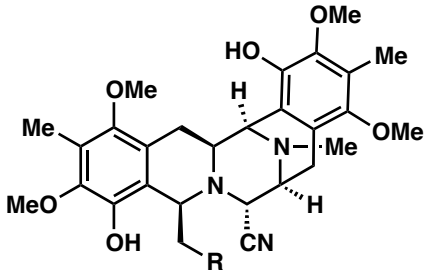
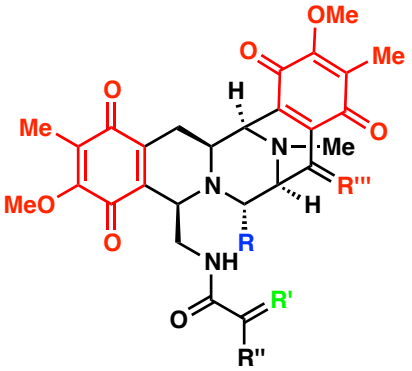
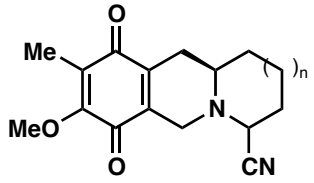


Saframycin analogs (Myers et al.)

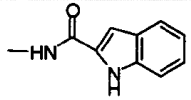
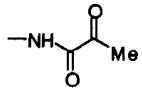
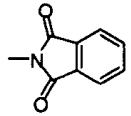
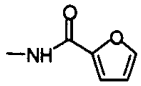
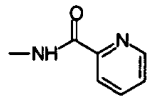
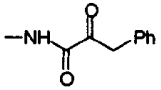
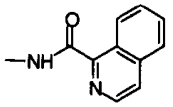
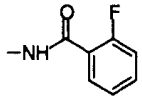
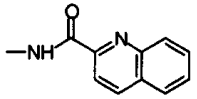
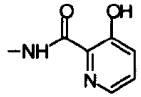
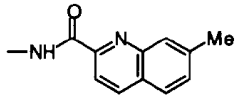
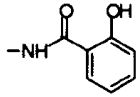
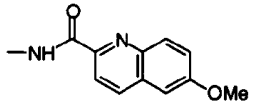
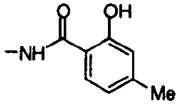
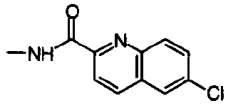
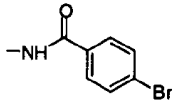
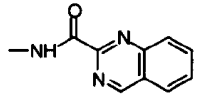
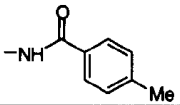
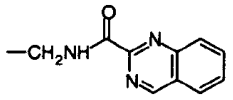


Modifications affecting overall, antiproliferative, and antimicrobial activity



n = 0, 1

No significant activity (Kubo et al.)

R^2 =	IC_{50} (nM) A375	A549	R^2 =	IC_{50} (nM) A375	A549
Saframycin A (3)	5.3	133		2.7	31
	4.5	160		1.7	9.2
	13	290		3.3	40
	2.4	39		2.5	32
	2.5	37		1.3	4.4
	1.4	14		1.4	4.6
	1.2	11		2.0	3.5
	1.2	6.5		1.5	4.1
	1.7	25		1.2	4.7
	1.9	37		3.6	78

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Ecteinascidins

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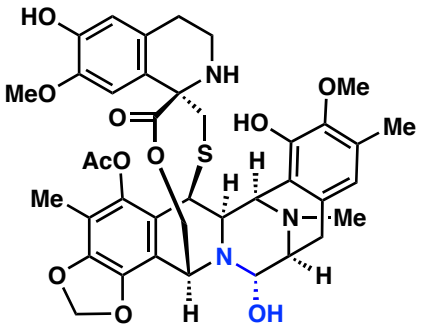
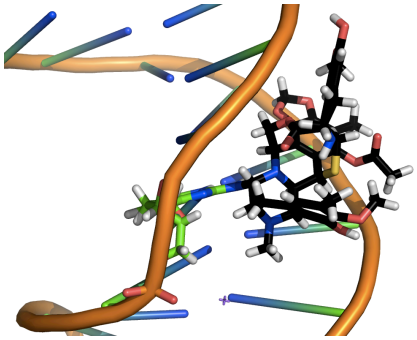


Ecteinascidin 743
(Trabectedin)



Modeled covalent complex
with DNA duplex fragment

Isolated in 1990 by Rinehart and co-workers from *Ecteinascidia turbinata*
13 members are know to date

Structure determined in 1990s by NMR, X-ray (derivatives), and degradation

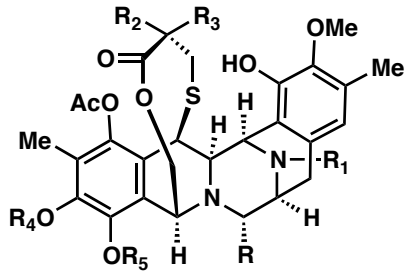
Three tyrosines, one cysteine, and six methionines are utilized in biosynthesis
Oxidation of tyrosine units is carried out by heme peroxidase, subsequent assembly of polycyclic core is carried out by NPRS system

Orders of magnitute more potent than saframycins
Potent antiproliferative activity (IC_{50} = 0.26, 0.34, 0.46, 0.5, 0.66 nM
for A549, P388, HT29, MEL-28, and L1210)
Potent antitumor activity (167% incresed lifespan at 15 μg/kg *vs* control mice,
P388 xenografts, up to 214% at 3.8 μg/kg for
related ecteinascidins)

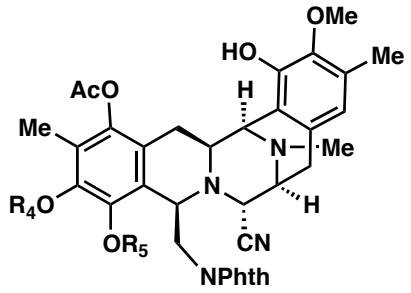
Alkylates minor groove similar to saframycins (1990s)
Forms DNA-Topo I cross-links (Corey, 1999; Hurley, 1999)
Disrupts DNA excision repair (Hurley, 2001)
DNA complexes bind GAPDH similar to saframycins (Myers, 2004)

Approved in Europe for soft tissue sarcoma (2007) and ovarian cancer (2009)
Marketed by Zeltia and Johnson and Johnson (as Yondelis)

Rejected by FDA in 2009 (combo with Doxil); in 2011 J & J pulled its submission



Ecteinascidins



Phthalascidin (Corey, 1999)

Ecteinascidins

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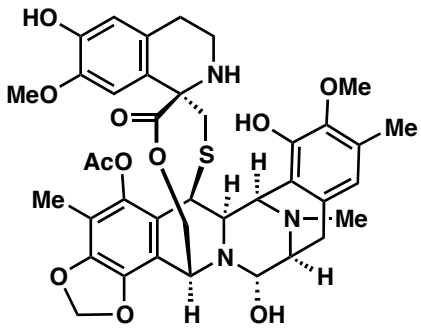
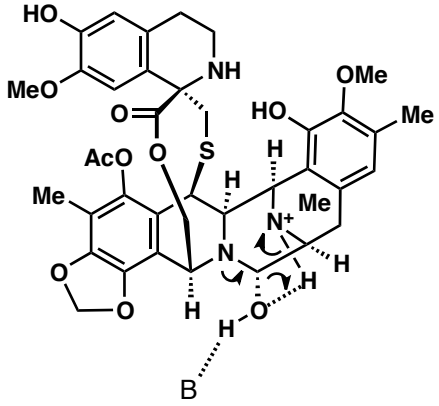
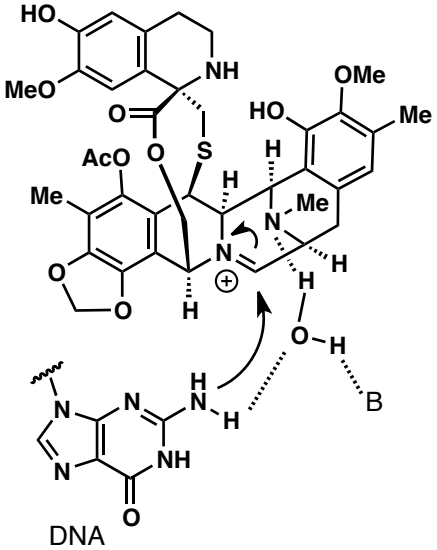
Shenvi Group

Hurley's proposal to address the role of tertiary amine (1998)

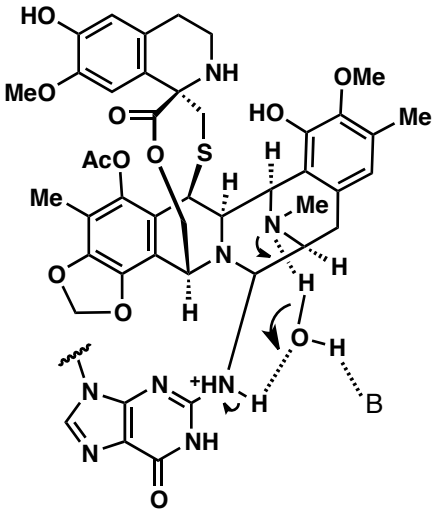


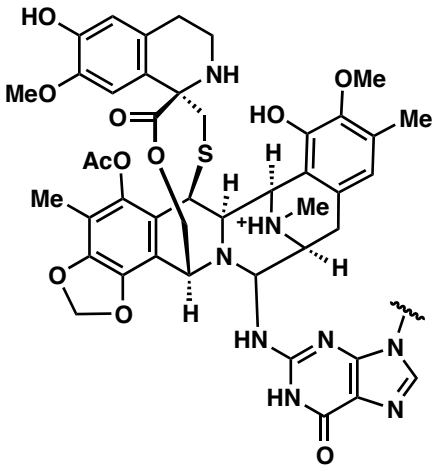
Ecteinascidin 743
(Trabectedin)





DNA





However, DNA alkylation alone doesn't explain extreme potency. Neither do DNA-Topo I cross-linking and DNA excision repair halting

GAPDH could be one of several targets

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March 8, 2012

Ecteinascidins

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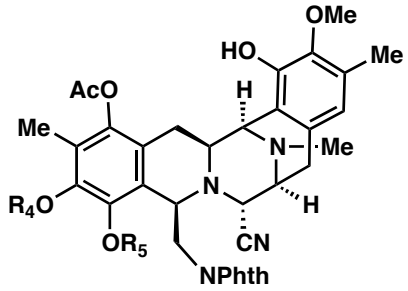
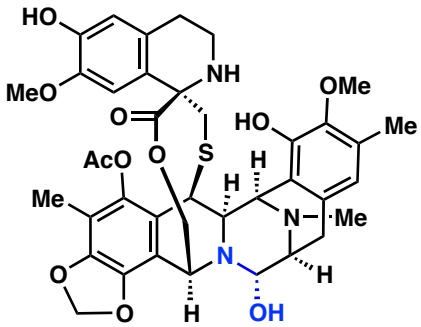
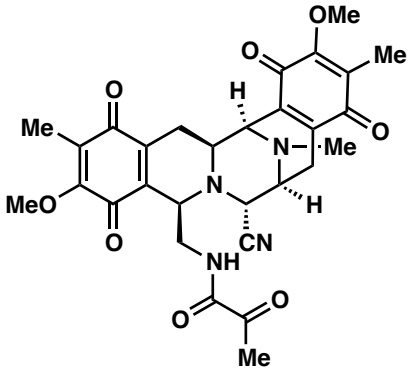


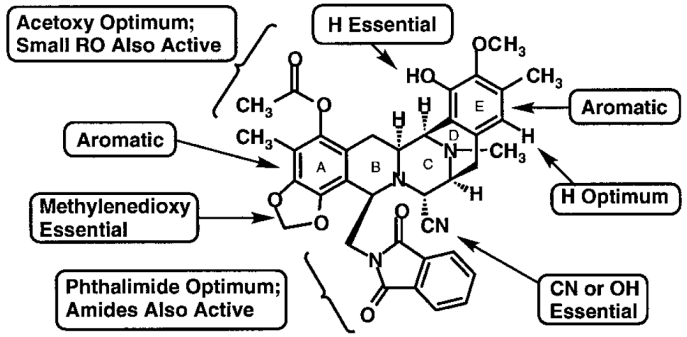
Phthalascidin (Corey, 1999)

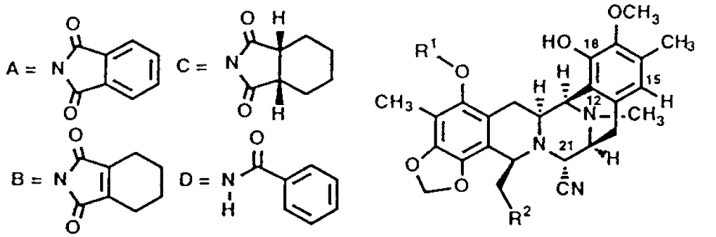


Ecteinascidin 743 (Trabectedin)



Saframycin A





			Cell line			
Analog	R^1	R^2	A-549	HCT116	A375	PC-3
Pt 650	$CH_3C(O)$	A	0.95	0.38	0.17	0.55
1	$CH_3C(O)$	D	3.2	0.59	0.35	0.64
2	$CH_3C(O)$	C	1.5	0.85	0.27	1.1
3	$CH_3C(O)$	B	1.2	0.61	0.35	0.75
4	$CH_3OCH_2C(O)$	A	1.6	0.87	0.31	0.90
5	$CH_3S(O)_2$	A	1.7	0.58	0.29	0.86
6	$CH_3CH_2C(O)$	A	2.1	1.2	0.51	2.9
7	CH_3	A	3.1	1.4	0.55	3.1
8	CH_3CH_2	A	3.9	1.7	0.97	2.4
C15-Chloro-Pt 650			2.8	1.0	0.35	1.1
C15-, C18-*p*-Quinone-Pt 650			1.7	0.80	0.45	0.68
N12-[^{14}C]-CH_3-Pt 650			0.91	—	0.22	—
C21-OH-Pt 650			1.0	0.61	0.20	0.53
C21-H-Pt 650			2200	1100	610	850
C18-OMOM-Pt 650			230	74	66	98
Et 743			1.0	0.50	0.15	0.70

Values reported are IC_{50} in nM.

Naphthyridinomycin, Cyanocyclines, and Bioxalomycins

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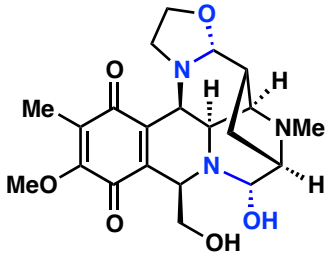


Naphthyridinomycin

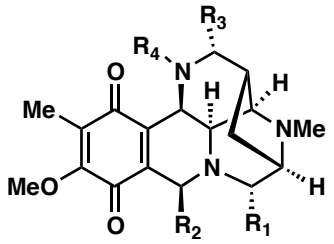
Naphthyridinomycin was isolated in 1974 by Kluepfel and co-workers from *Streptomyces lusitanus* (unstable red solid)
Cyanocycline A was isolated in 1982 by Hayashi and co-workers
11 members are know to date

X-ray analysis of naphthyridinomycin (1975) and cyanocycline A (1983)

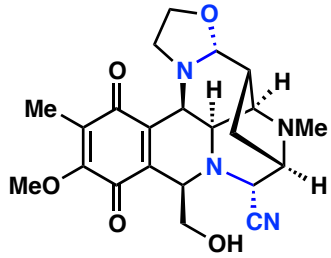
Tyrosine, glycine, ornithine, and methionine are incorporated

Potent antimicrobial activity (MICs below 0.025 μg/mL)

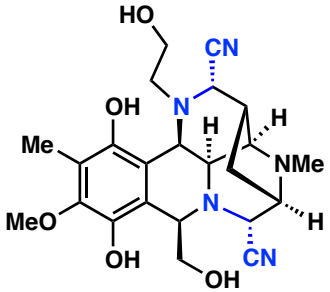
Inhibition of bacterial DNA synthesis, alkylation of DNA



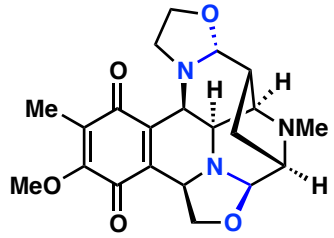
Cyanocyclines, bioxalomycins



Cyanocycline A



Cyanocycline D



Bioxalomycin β_2

Naphthyridinomycin, Cyanocyclines, and Bioxalomycins

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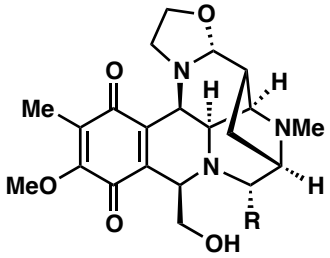
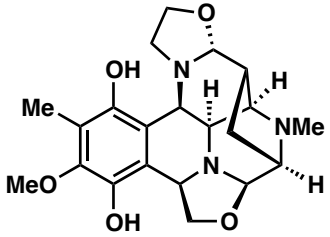


Naphthyridinomycin (R = OH)
Cyanocycline A (R = CN)

Bacteria	Minimum inhibitory concentration (μg/ml)
Staphylococcus aureus pen^S	<0.025
Staphylococcus aureus pen^R	<0.025
Streptococcus faecalis	<0.025
Escherichia coli	0.8
Enterobacter aerogenes	0.2
Salmonella pullorum	0.2
Pseudomonas aeruginosa	0.2
Pseudomonas fluorescens	1.6
Proteus mirabilis	0.4
Proteus vulgaris	0.4
Klebsiella pneumoniae	0.05
Serratia marcescens	0.05

Bacteria	NAPA	CYANO
Staphylococcus aureus	0.012	0.047
Streptococcus enterus	>1.5	>1.5
Escherichia coli	0.375	0.75
Proteus vulgaris	1.5	1.5
Serratia marcescens	0.75	>1.5
Klebsiella pneumoniae	1.5	1.5



Bioxalomycin α_2

Organism [no. of strains]	MIC (μg/ml)		
	Bioxalomycin α2	Piperacillin	Vancomycin
MSSA [4]	≤0.002~0.015	1~4	1
MRSA [33]	0.004~0.015	>128	1~2
SCN [6]	≤0.002~0.004	1~>128	1~2
Staphylococcus hemolyticus [1]	≤0.002	>128	1
Streptococcus pyogenes [1]	≤0.002	≤0.06	0.25
Streptococcus agalactiae [1]	≤0.002	≤0.06	0.50
Streptococcus pneumoniae [1]	0.015	≤0.06	0.25
Enterococcus faecalis VS [4]	≤0.002~0.25	1~>128	0.50~1
Enterococcus faecium VR [2]	0.03~0.06	128~>128	>128
Bacillus cereus [1]	0.12	2	1

Naphthyridinomycin, Cyanocyclines, and Bioxalomycins

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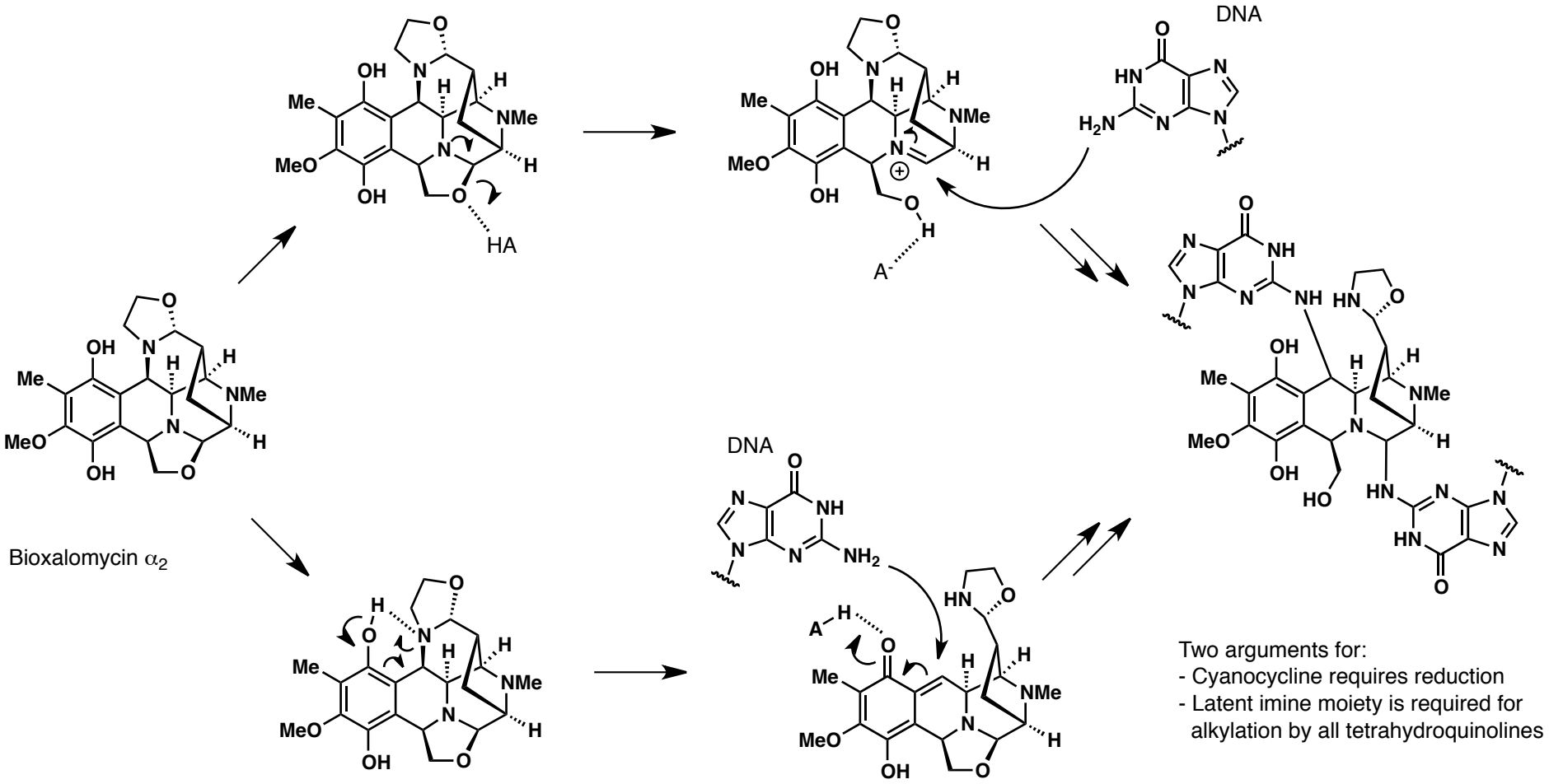
DNA duplex cross-linking observed by Williams and co-workers in 1998: new mechanism proposed



Two arguments for:
- Cyanocycline requires reduction
- Latent imine moiety is required for alkylation by all tetrahydroquinolines

Quinocarcin

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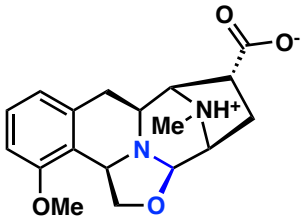
Shenvi Group



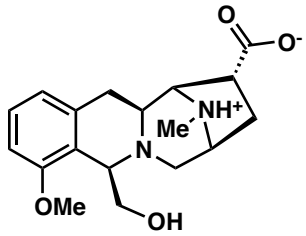
Quionocarcin

Isolated in 1983 by Tomita and co-workers from *Streptomyces melanovinaceus*

Structure determined in 1983 by X-ray (quinocarcinol), NMR, and degradation
Absolute stereochemistry determined by synthesis (Garner,1992)

Weakly active in bacterial assays (MICs 12-25 μg/mL)
DNA polymerase inhibitory activity assigned
Targets and covalently modifies radixin (Fenteany, 2006)

Significant antiproliferative activity (IC_{50} = 110 nM for P388)
RNA synthesis affected in P388 cells
Potent antitumor activity against P388 tumor zenograft studies



Quionocarcinol

The effects of dose and schedule on the efficacy of KW2152 against P388 leukemia were determined. P388 cells (1 × 10^6/mouse) were implanted into $CD2F_1$ mice (6 mice/group) on day 0 and i.p. treatment was initiated on day 1 according to the schedules shown below.

	Day 1		Days 1, 5, 9		Days 1–7	
Dose (mg/kg/day)	Mean survival days ± SD	T/C (%)	Mean survival days ± SD	T/C (%)	Mean survival days ± SD	T/C (%)
Control	10.0 ± 0.8		10.0 ± 0.8		10.0 ± 0.8	
24.6	4.5 ± 3.8	45	NT[a]		NT	
16.4	13.0 ± 3.5	130	NT		NT	
12.3	NT		6.2 ± 0.4	62	NT	
8.2	11.8 ± 1.1	118	17.0 ± 0	170	19.8 ± 8.9	198
6.1	NT		15.3 ± 1.8	153	21.5 ± 2.5	215
4.1	11.7 ± 0.8	117	15.3 ± 1.2	153	19.2 ± 1.3	192
2.1	11.7 ± 0.5	117	13.8 ± 1.7	138	18.0 ± 2.3	180
1.0	NT		12.5 ± 1.0	125	15.3 ± 1.4	153
0.51	NT		12.0 ± 1.6	120	13.8 ± 2.3	138
0.26	NT		NT		13.0 ± 1.2	130

P388, P388/MMC, and P388/ADM cells (1 × 10^6/mouse) were implanted into $CD2F_1$ mice on day 0 (6 mice/group). Treatment was initiated on Day 1.

Tumor	Tumor site	Drug	Drug route	Schedule of treatment days	Dose (mg/kg/day)	T/C (%)
P388	i.p.	KW2152	i.p.	1	7.1	132
			i.p.	1–7	5.4	198
		MMC	i.p.	1	6.0	164
		ADM	i.p.	1	12.0	215
P388/MMC	i.p.	KW2152	i.p.	1	7.1	111
			i.p.	1–7	5.4	168
		MMC	i.p.	1	2.0	104
P388/ADM	i.p.	KW2152	i.p.	1	14.3	124
			i.p.	1–7	3.6	168
		ADM	i.p.	1	8.0	108

Quinocarcin

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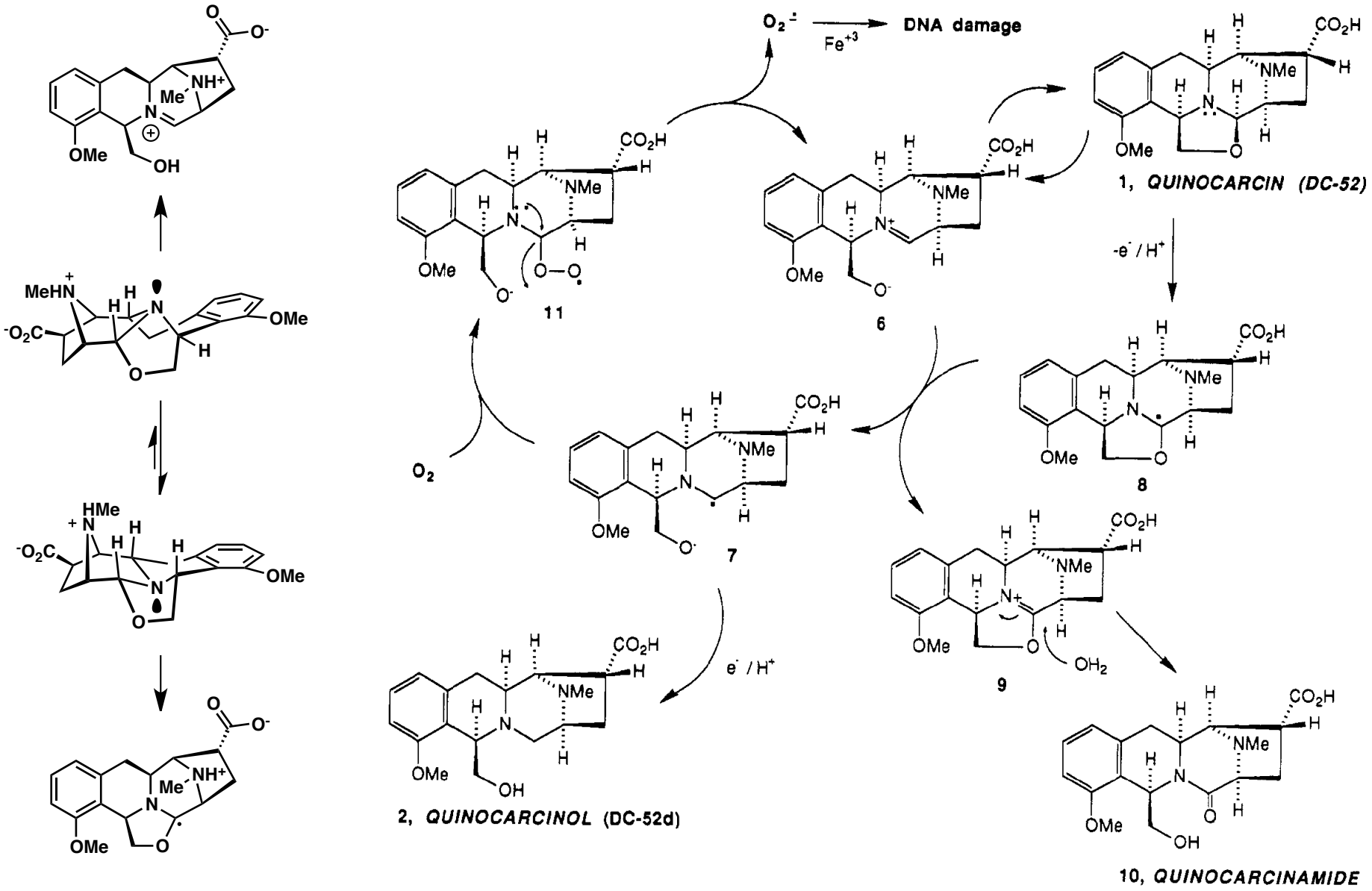
Molecular basis for DNA cleavage mechanism according to Williams (1992)



Tetrahydroquinoline Natural Products: Synthetic Work

Sergey Pronin
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Saframycins

Fukuyama, **1982**
Kubo, **1987**
Fukuyama, **1990**
Myers, **1999**
Corey, **1999**
Liu, **2011**

Tetrazomine

Williams, **2001**

Esteinacidins

Corey, **1996**
Cuevas, **2000**
(semisynthesis)
Fukuyama, **2002**
Zhu, **2006**
Danishefsky, **2006**

Cyanocylclines

Evans, **1986**
Fukuyama, **1986**
Fukuyama, **1986**
(bioxalomycin?)
Garner, **2007**

Renieramycins

Fukuyama, **1990**
Magnus, **2005**
Williams, **2005**
Zhu, **2007**
Williams, **2007**
Liu, **2009**
Zhu, **2009**
Saito, **2011**
Williams, **2011**

Quinocarcin(ol)

Danishefsky, **1985** (ol)
Fukuyama, **1988**
Garner, **1992**
Terashima, **1993**
Myers, **2005**
Zhu, **2008**
Stoltz, **2008**

Jorumycin

Williams, **2005**
Zhu, **2009**
Liu, **2012**

Lemonomycin

Stoltz, **2003**

Cyclic Imine Phycotoxins

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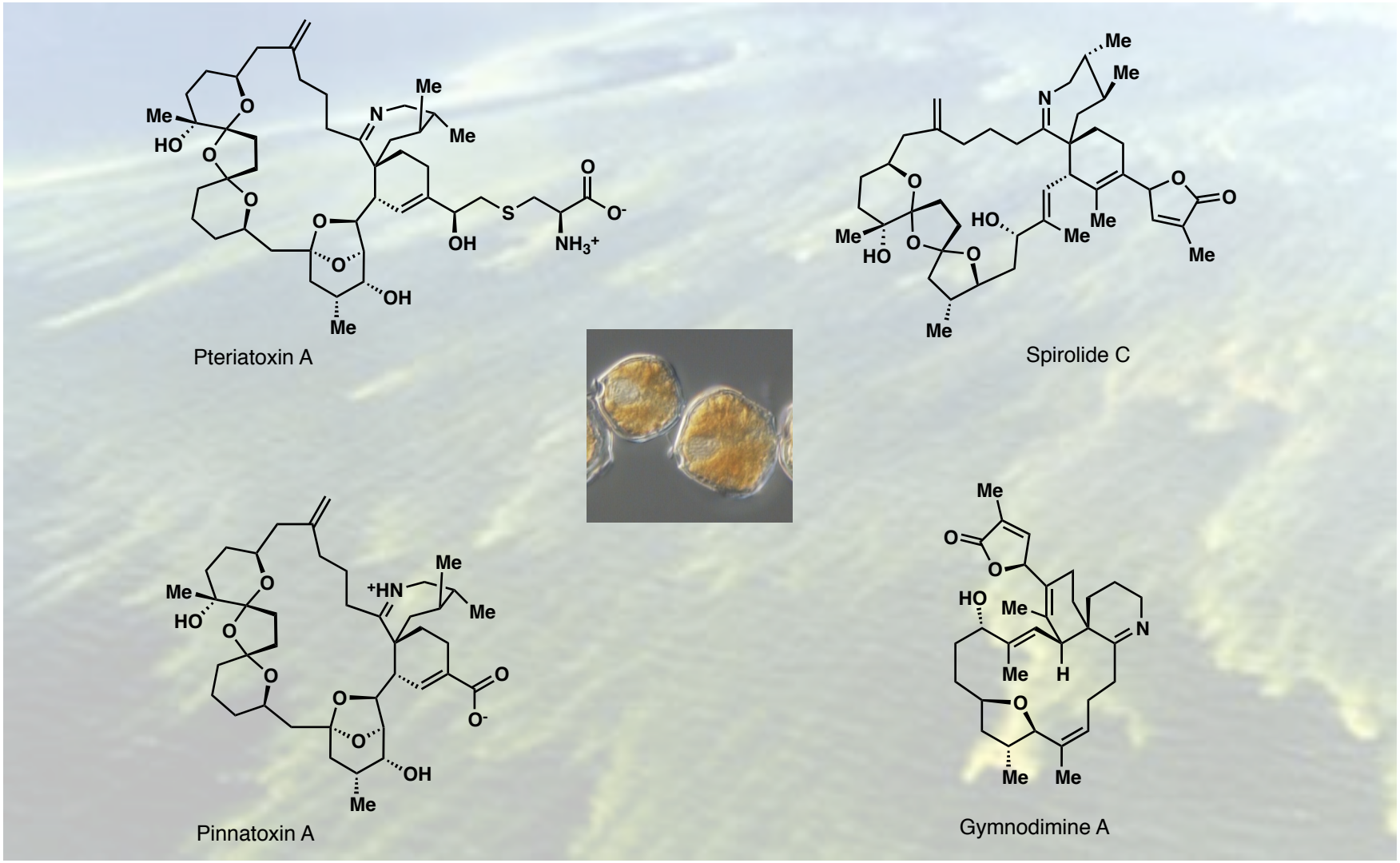

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Spirolides

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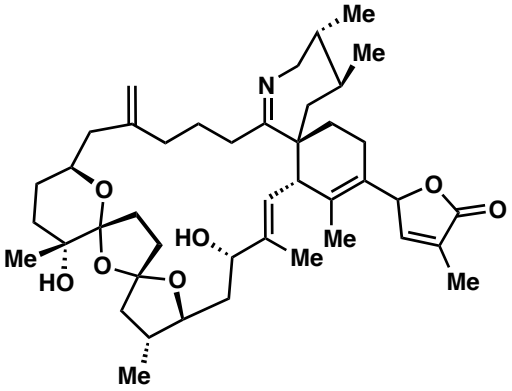


Spirolide C

Isolated in 1995 by Hu and co-workers from shellfish extracts
Origin: dinoflagellate *Alexandrium ostenfeldii*
14 members know to date

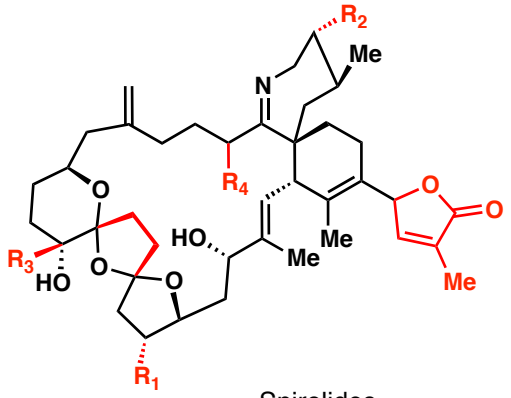
Relative stereochemistry assigned only in 2009 (Ciminiello et al.)

Importance of cyclic imine moiety was realized as early as 1996

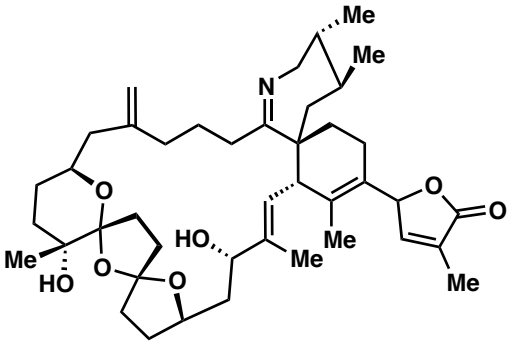
Extremely potent fast-acting neurotoxin: 75 μg/kg kills mouse in 8 min
Similar toxicity profiles for all imine-containing spirolides

Mode of action involves inhibition of nAChRs (Kharrat et al., 2008)

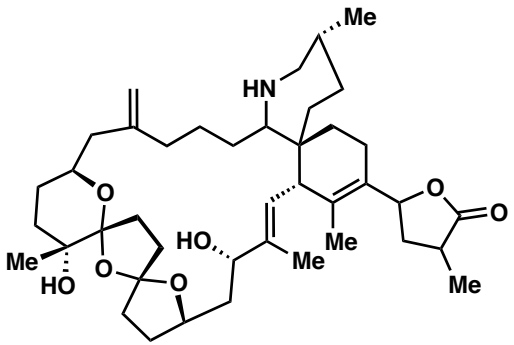
Binds nACh receptors with extremely high potency
K_i down to 80 pM, IC_{50} down to 0.5 nM
X-ray structure of complexes with nAChBP of *Aplysia californica* solved in 2009 (Bourne, Marchot)



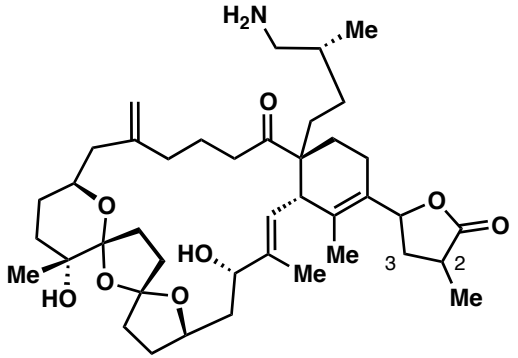
Spirolides



13-desMe Spirolide C



reduced Spirolide B (large drop in toxicity)



Spirolides E ($\Delta^{2,3}$), F (non-toxic)

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Gymnodimines

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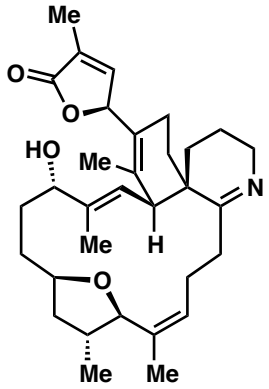
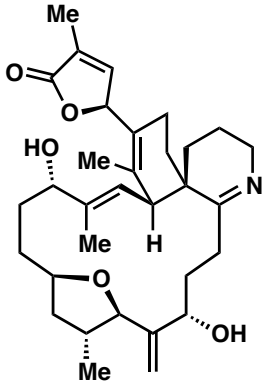
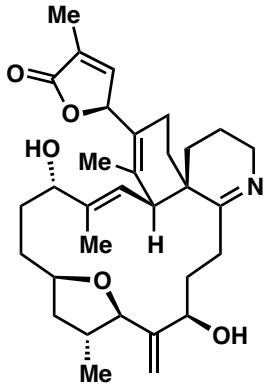


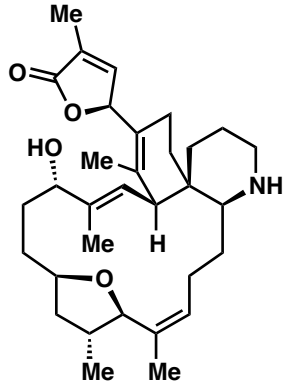
Gymnodimine A



Gymnodimine B



Gymnodimine C



Gymnodamine

First isolated in 1995 by Yasumoto and co-workers from oysters
Producing organism: dinoflagellate *Gymnodinium selliforme*

Structure elucidated by NMR and confirmed by X-ray analysis of the derivative of gymnodamine (Blunt, 1997)

Potent neurotoxin: lethal dose for mice 0.45 mg/kg

Other members were isolated from the same organism (Miles; 2000, 2003)

Orignally, action on Na^+-channels was suggested (Seki, 1996) but later shown to block nACh receptors (Molgo, 2008)

X-ray structure of complexes with nAChBP of *Aplysia californica* solved in 2009 (Bourne, Marchot)

nAChBP Complexes

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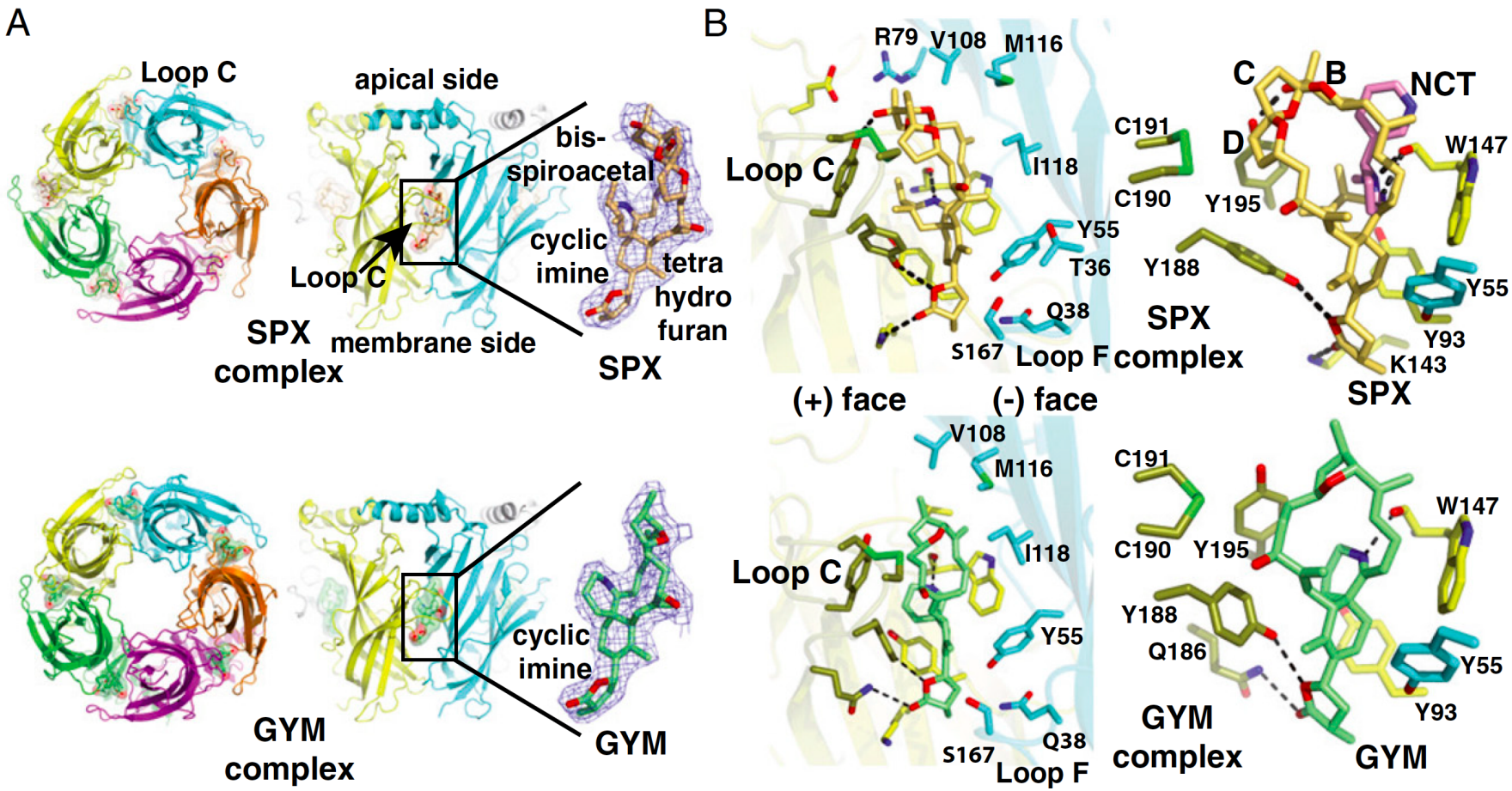

13-desMe spirolide C (SPX) and gymnodimine (GYM) bound to *Aplysia californica* nAChBP (Bourne, Marchot; 2010)

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Pinnatoxins and Pteriatoxins

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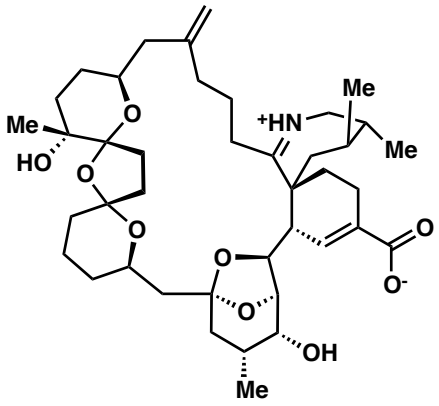
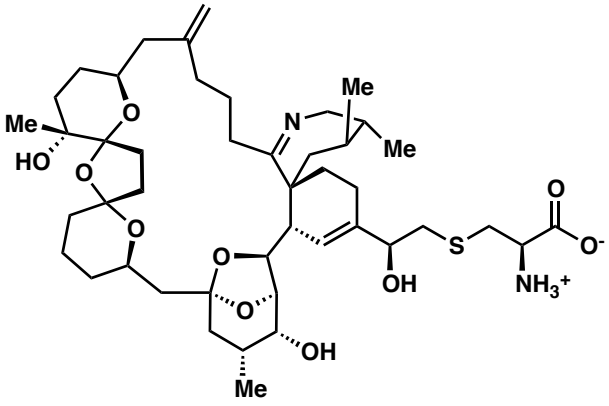


Pinnatoxin A

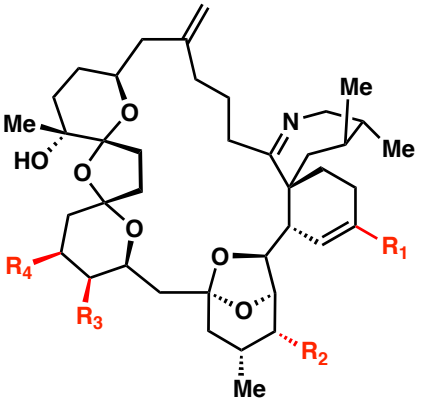
Pinnatoxin A was isolated in 1995 by Uemura and co-workers from shellfish *Pinna muricata*
Producing organism: peridinoid dinoflagellate (2010)
6 members discovered to date

Structure elucidation was complete with Kishi's synthesis in 1998

Extremely potent neurotoxin: LD_{50} as low as 10 μg/kg in mice
Originally was believed to activated Ca^{+2}-channels
Mode of action revised in 2011 in favor of inhibition of nACh receptors



Pteriatoxin A

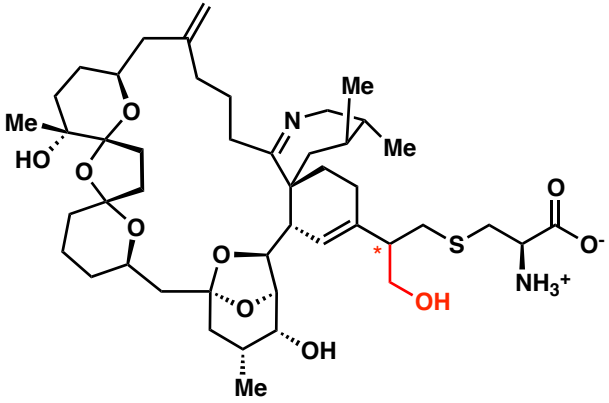


Pinnatoxins

Pteriatoxins were isolated in 2001 by Uemura and co-workers from shellfish *Pteria penguin*

Structure elucidation was complete with Kishi's synthesis of all pteriatoxins in 2006

Extremely potent neurotoxin: LD_{99} as low as 8 μg/kg in mice



Pteriatoxins B (*R*), C (*S*)

Pinnatoxins and Pteriatoxins

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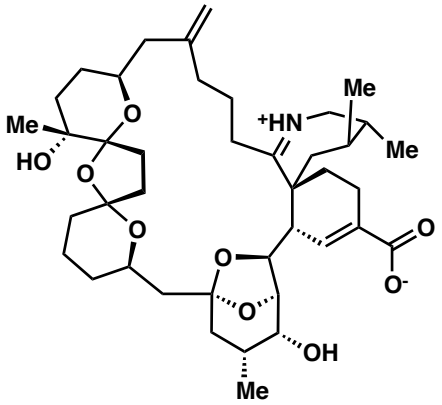
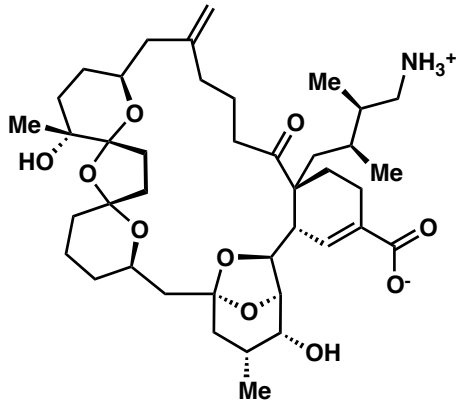


Pinnatoxin A



Pinnatoxin A amino acid

Table 3. Affinity Constants for PnTX A and Its Amino Keto Analogue (PnTX AK) on Muscle and Neuronal nAChR Subtypes[a]

	$K_i \pm$ SEM[b] (nM)			
ligand	$\alpha 1_2\beta\gamma\delta$ (*Torpedo*)	$\alpha 7-5HT_3$ (chick)	$\alpha 4\beta 2$ (human)	$\alpha 3\beta 2$ (human)
PnTX A	2.8 ± 0.03	0.35 ± 0.04	15.6 ± 5.2	9.4 ± 1.9
PnTX AK	>1000	>10 000	>2000	>2000

Zakarian confirmed the role of cyclic imine moiety in 2011 and suggested a role for electrostatic interactions

TSRI
March 8, 2012

Phycotoxins: Synthetic Work

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Gymnodimines

Romo, **2009**

Pinnatoxins

Kishi, **1998**
Hirama, **2004**
Kishi, **2006**
Hashimoto, **2008**
Zakarian, **2008**
Zakarian, **2011**

Pteriatoxins

Kishi, **2006**