



# *Synthesis and Pharmacological Activity of Prenyloxypheophenylpropanoids*

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## **OXYPRENYLATED SECONDARY METABOLITES**

- PRENYLOXYALKALOIDS
- PRENYLOXYPHENYLPROPANOIDS

ACETOPHENONES

ALCOHOLS AND ESTERS

ALDEHYDES

ANTHRAQUINONE

BENZOIC ACIDS

BENZOPHENONES

CHALCONES

CINNAMIC ACIDS

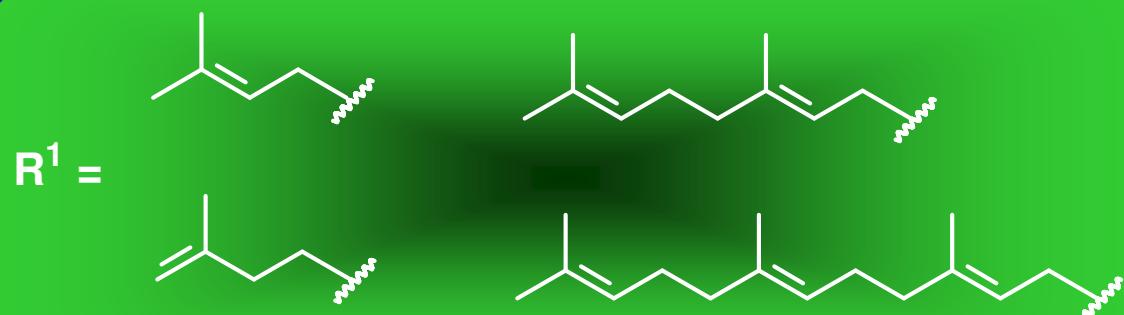
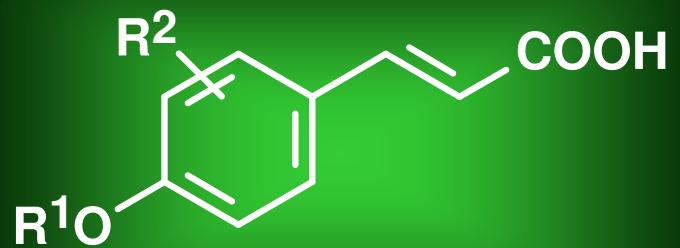
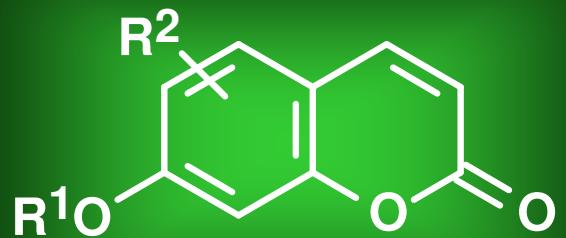
COUMARINS

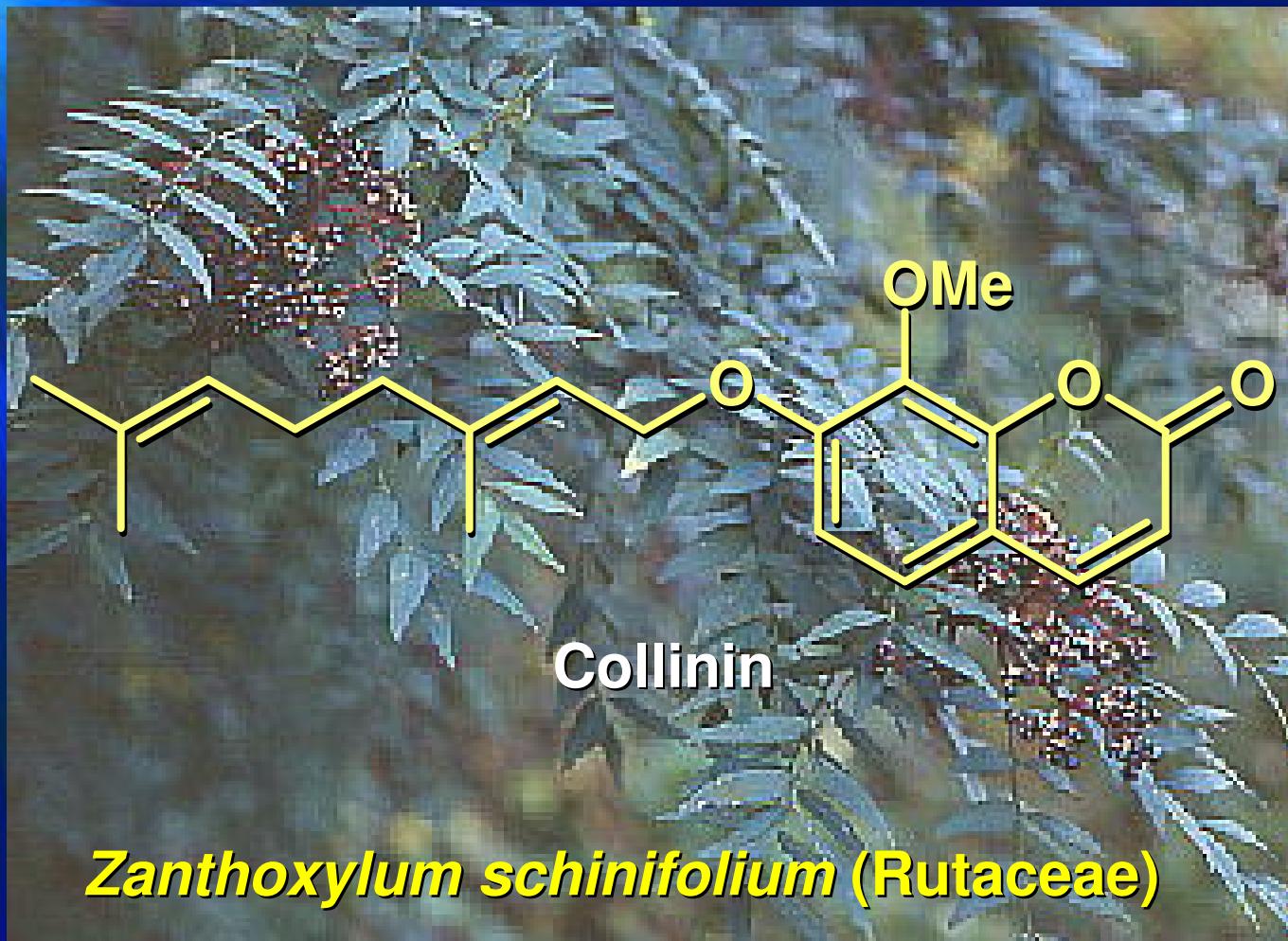
FLAVONOIDS

LIGNANS

TERPHENYL DERIVS.

XANTHONES

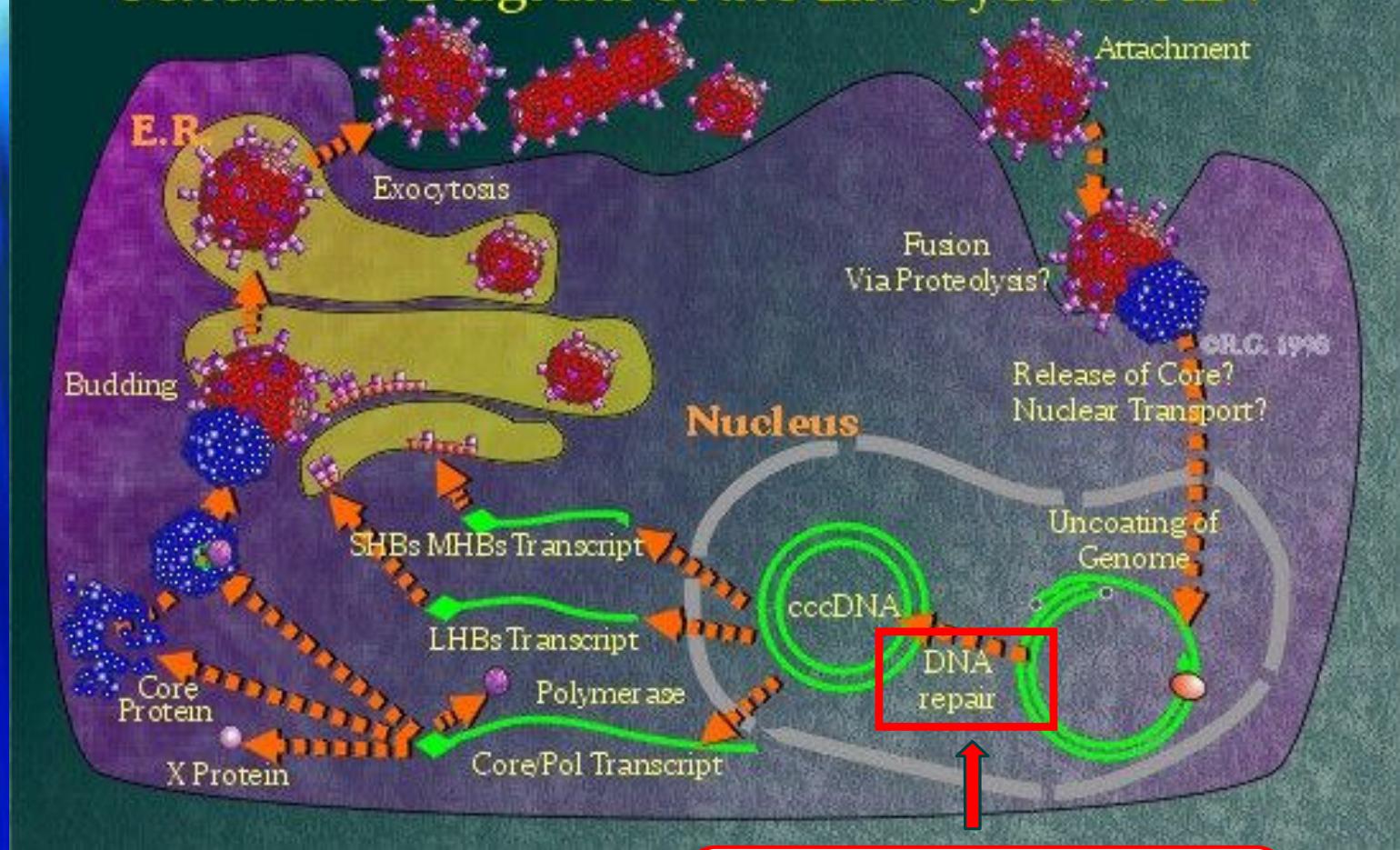




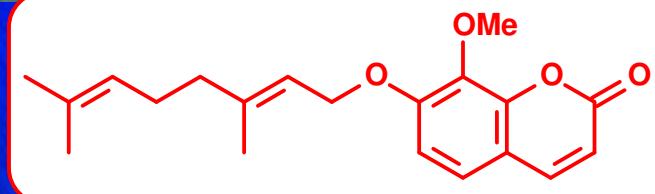
**Roots 0.00014 - 0.00027 %**

Chen, I.S.; Lin, Y.C.; Tsai, L.I.; Teng, C.M.; Ko, F.N.; Ishikawa, T.; Ishii, H. *Phytochemistry* 1995, 39, 1091

# Schematic Diagram of the Life Cycle of HBV

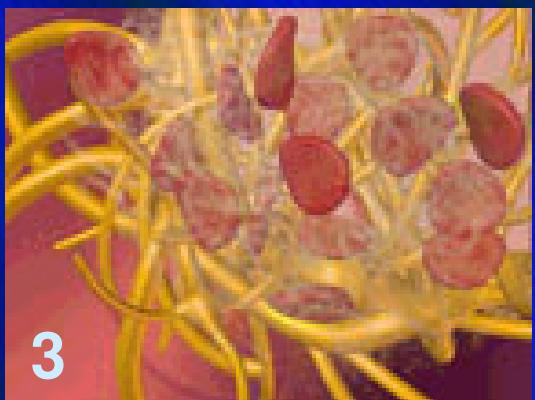
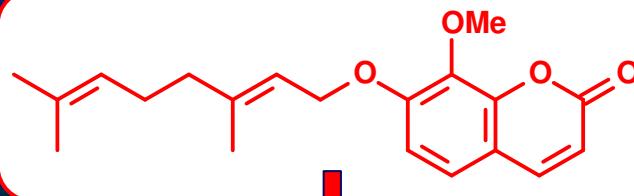


**IC<sub>50</sub> = 17.1 µg/mL**



Chang, C.T.; Doong, S.L.; Tsai, L.I.; Chen, I.S. *Phytochemistry* 1997, 45, 1419

# Blood coagulation



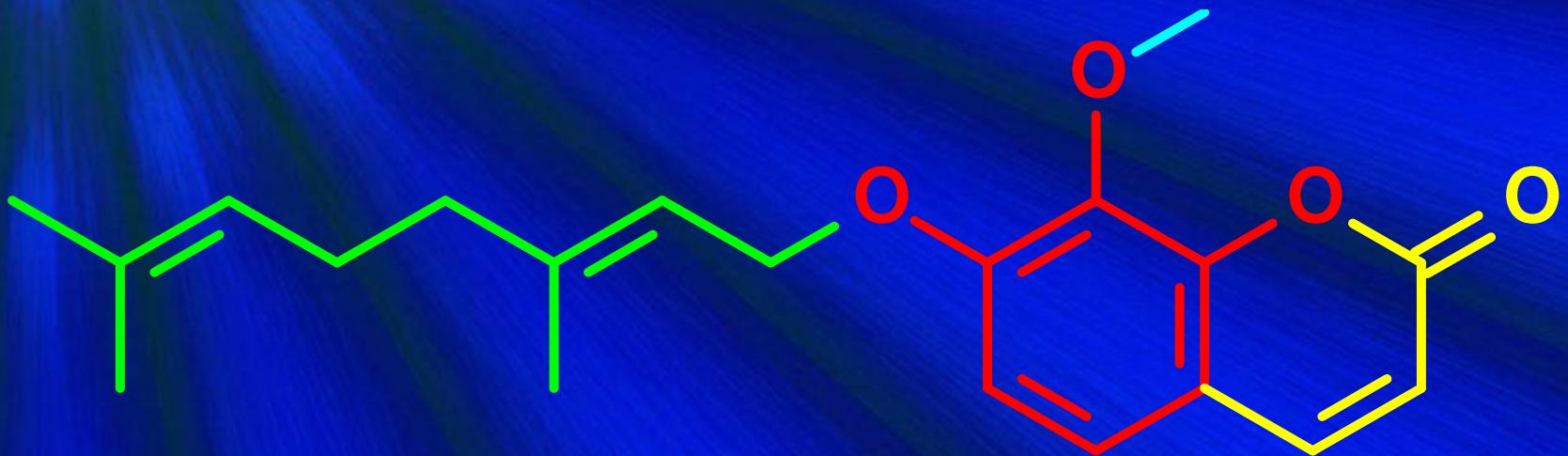
**50 µg/mL 100% inhibition of platelet aggregation**

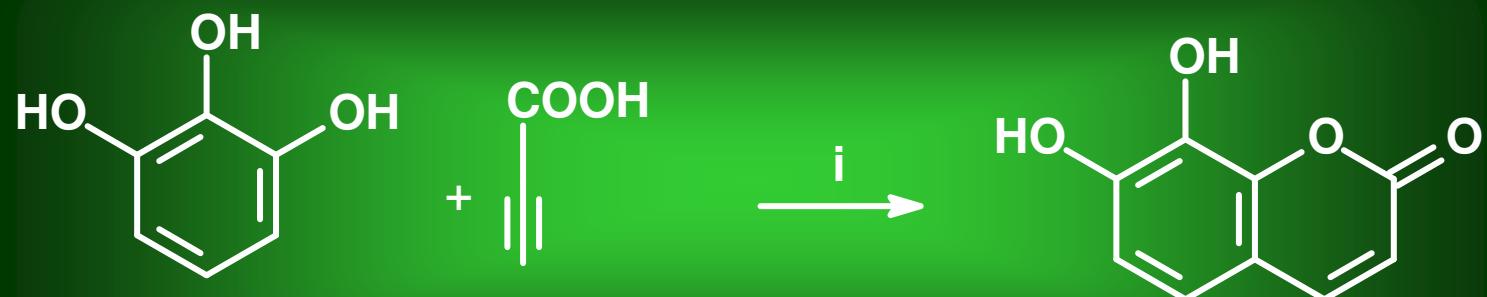


- Low yield of extraction from natural sources
- Few data on the pharmacological profile

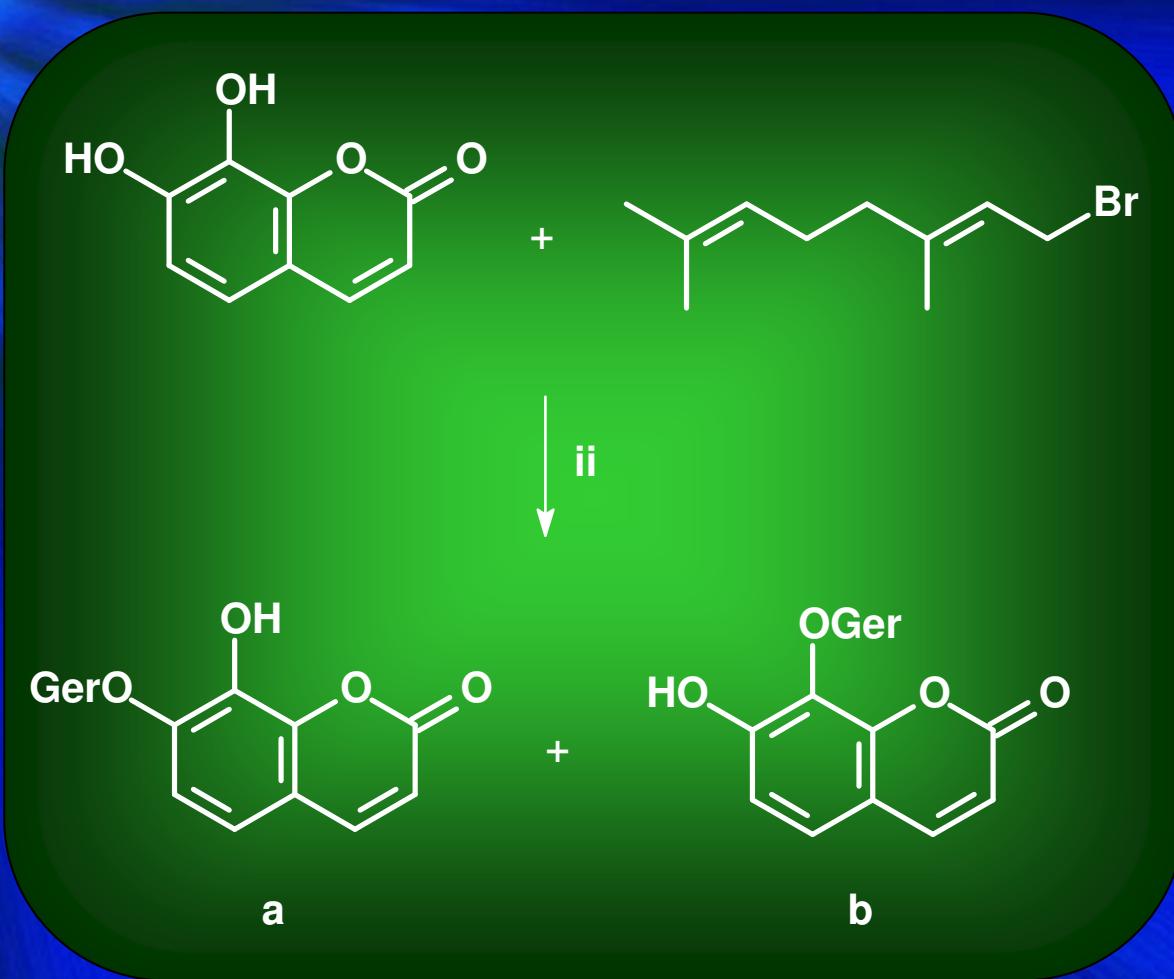


**SYNTHESIS**

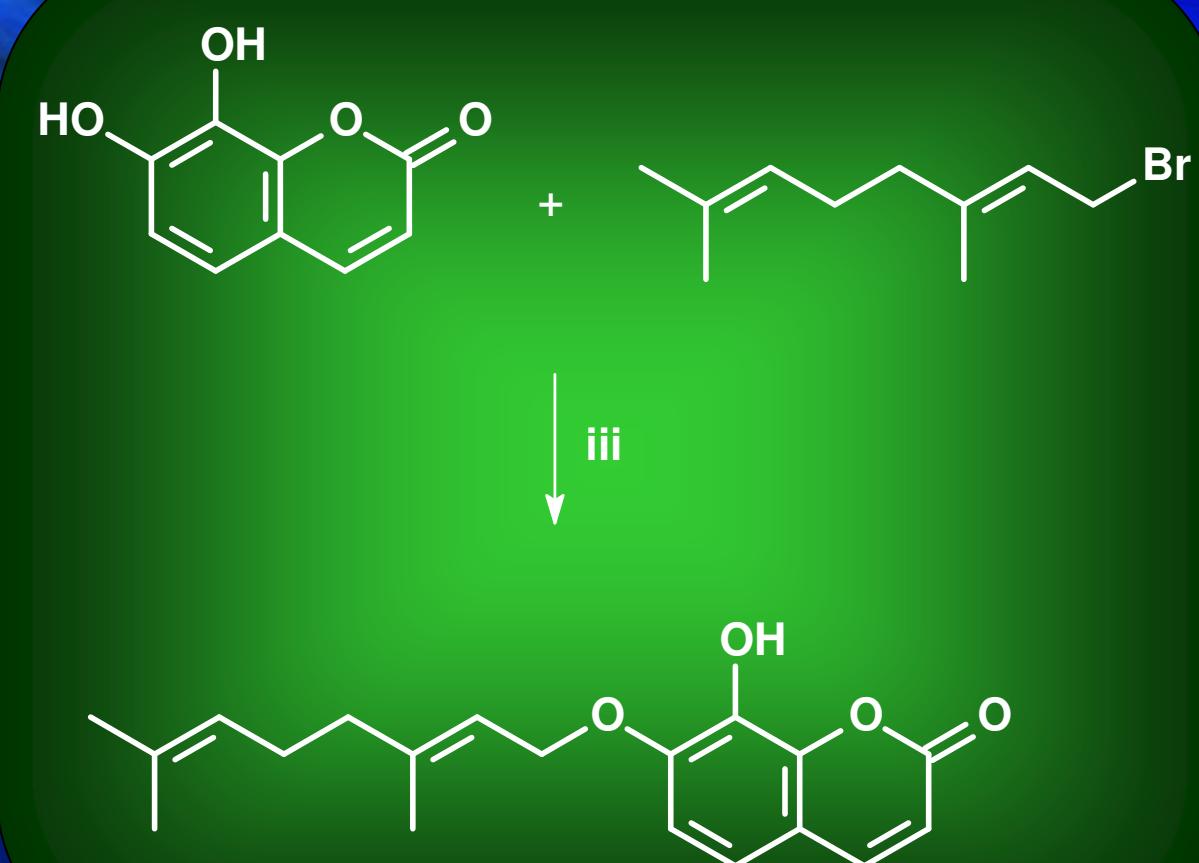




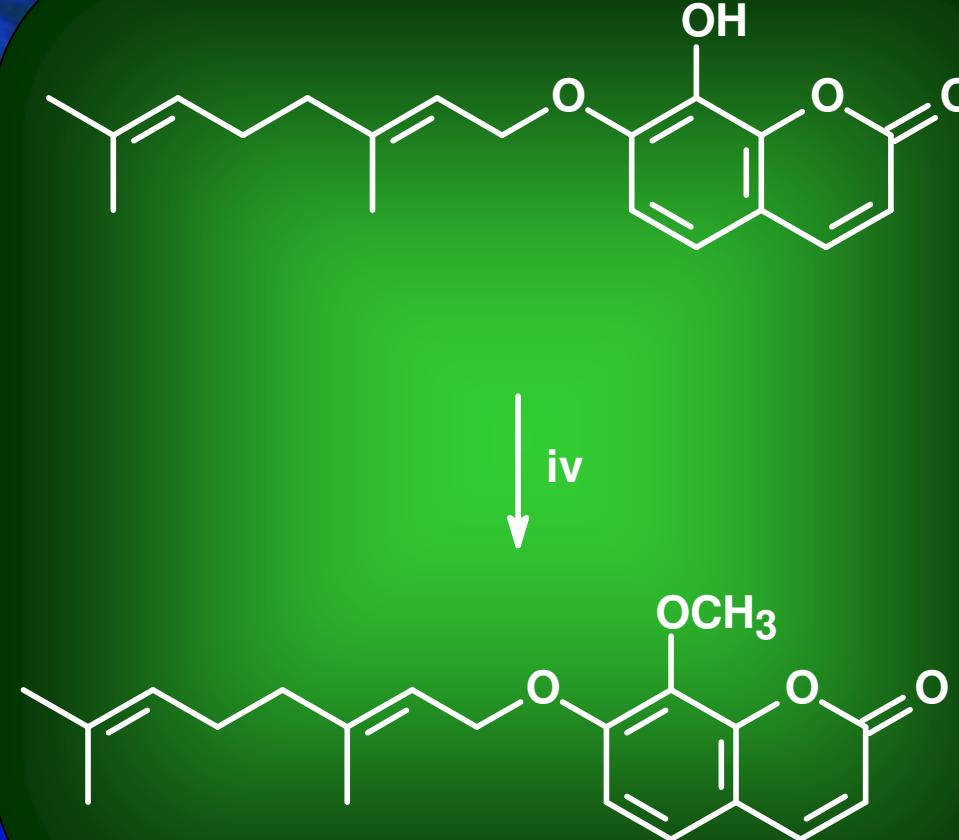
i:  $\text{H}_2\text{SO}_4$  conc. (cat.), 120 °C, 30 min., 59%



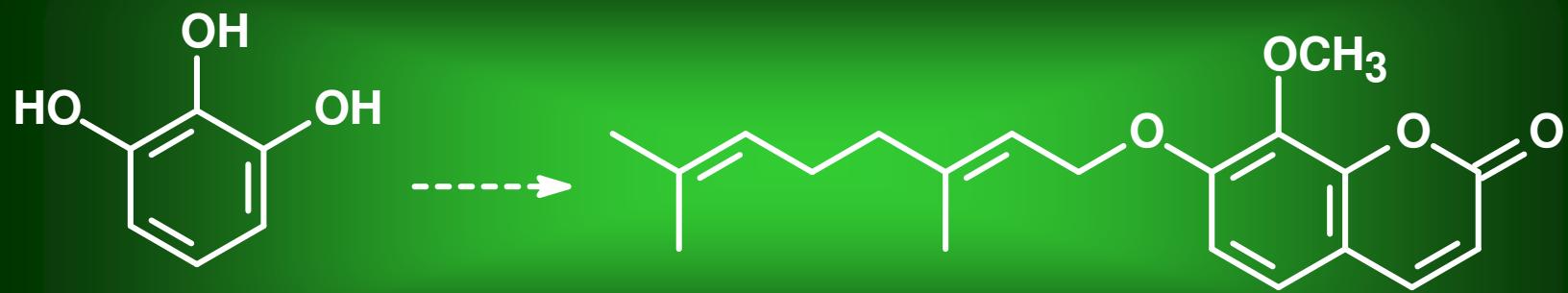
ii:  $\text{K}_2\text{CO}_3$ , acetone, 60 °C, 5h, a/b 1:1, 70%,  $\text{NaH}$ , THF, 0 °C, 3h, a/b 55:45, 74%



iii: DBU, acetone, r.t., 3h, 65%



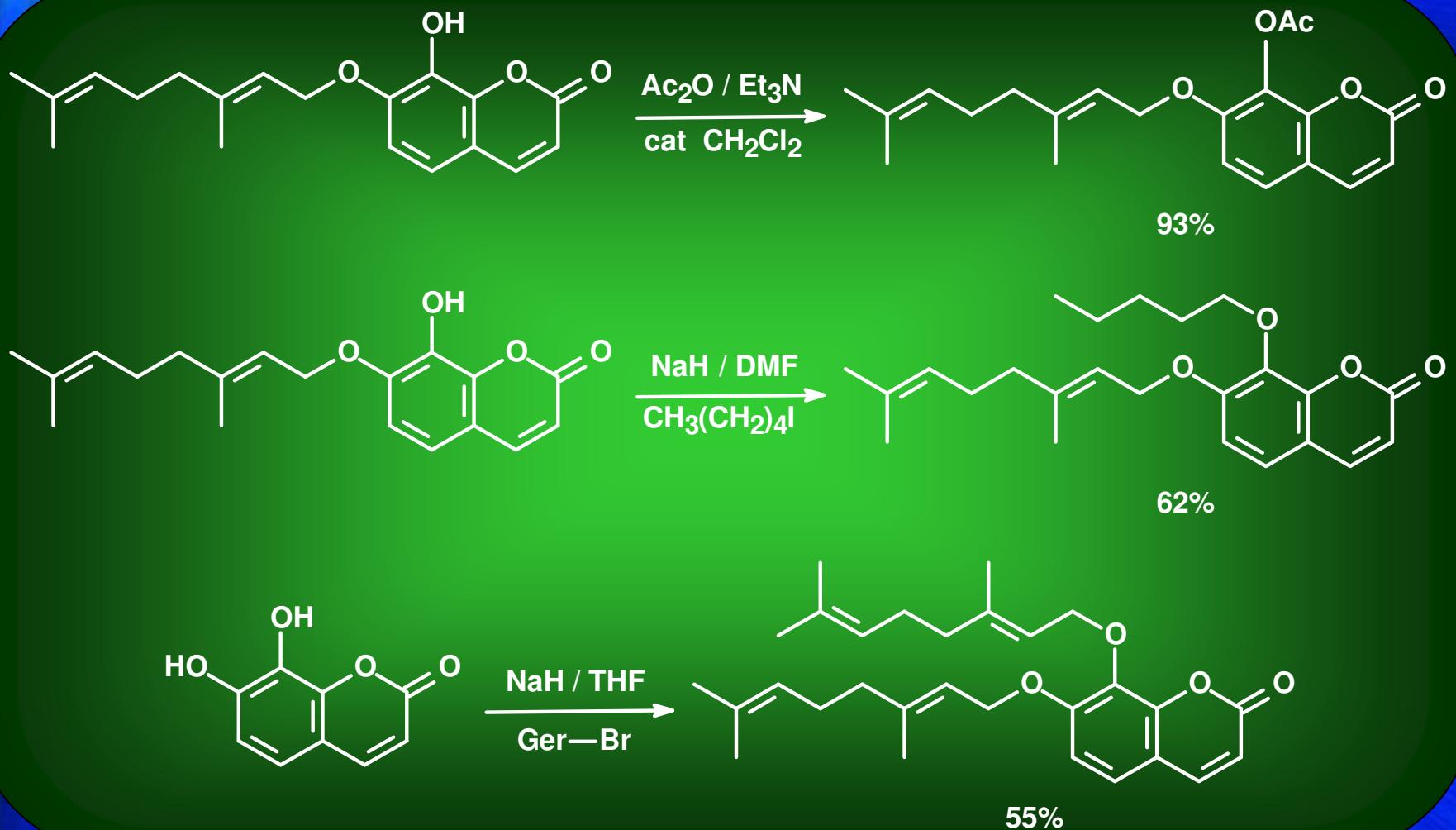
iv: CH<sub>3</sub>I, NaH, DMF, r.t., 2h, 64%



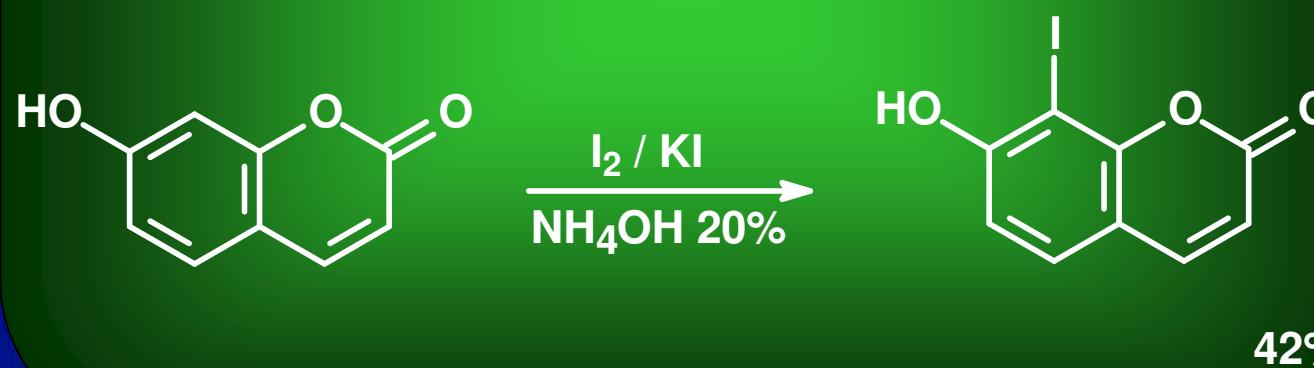
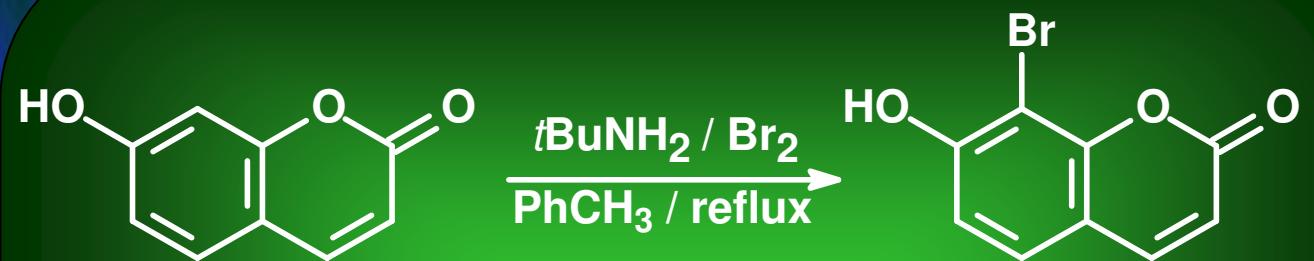
Overall yield 24.6 %

Curini, M.; Epifano, F.; Maltese, M.; Marcotullio, M.C.; Prieto Gonzales, S.; Rodriguez, J.C. *Aust. J. Chem.* 2003, **56**, 59

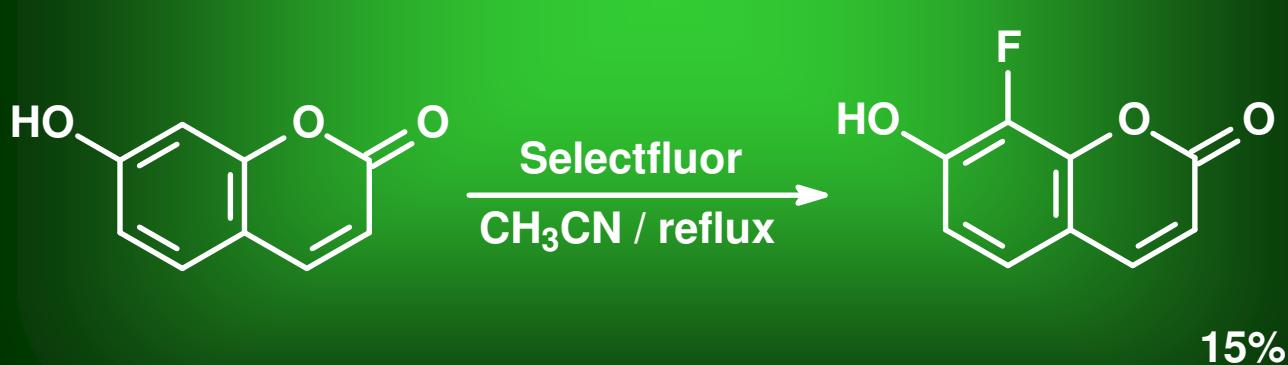
# Synthesis of 8-O-derivatives

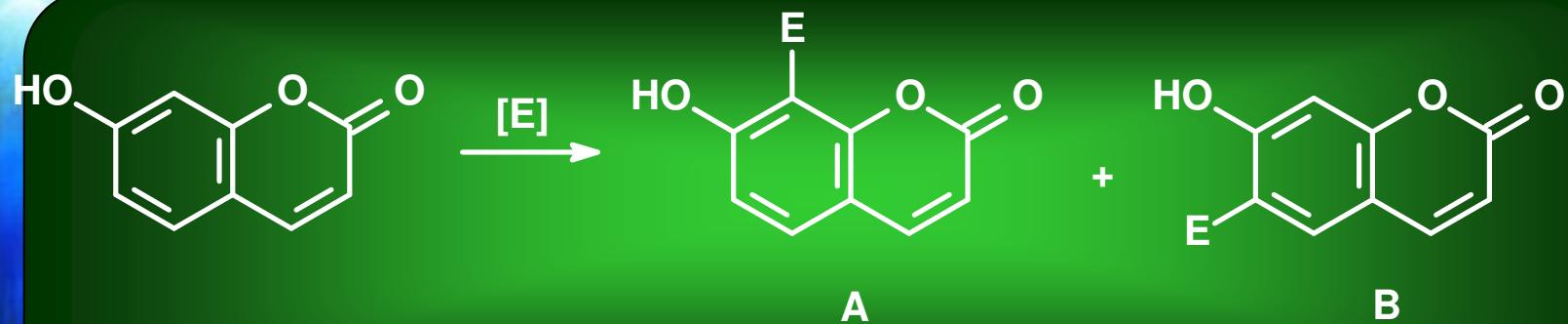


# Synthesis of 8-halo-7-hydroxycoumarins

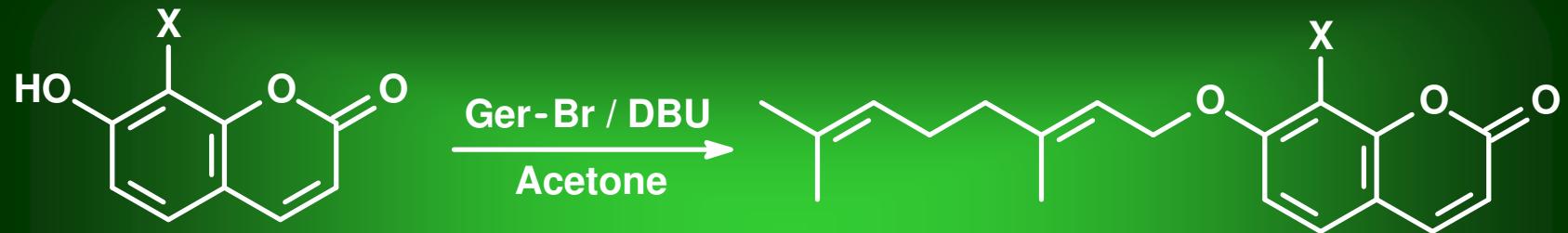


Harayama, T.; Nishita, Y. *Chem. Pharm. Bull.* 1996, 44, 1986

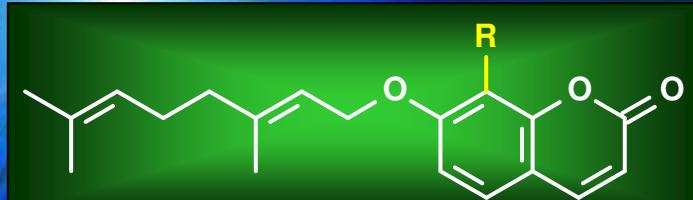




E	Temperature	A/B
I	25	100:0
Br	25	0:100
Br	110	50:50
Cl	25	0:100
Cl	100	30:70
F	65	15:85



X = -I 55%  
X = -Br 51%  
X = -Cl 60%  
X = -F 52%



## Anti-inflammatory activity Croton oil ear test



R = -H (auraptene)



R = -OH



R = -OAc



R = -O(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>



R = -OGer



R = -CH<sub>3</sub>



R = -I



R = -Br



R = -Cl



R = -F



Collinin



Indomethacin



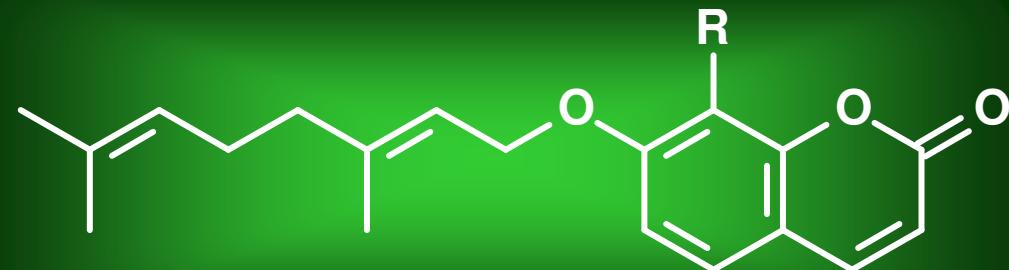
Controls

	Dose ( $\mu\text{mol}/\text{cm}^2$ )	Nr. animals	oedema (mg) $m \pm D.S.$	Red. %
R = -H (auraptene)	1.00	10	$3.4 \pm 0.2^*$	51
R = -OH	1.00	10	$4.3 \pm 0.2^*$	38
R = -OAc	1.00	10	$3.4 \pm 0.2^*$	51
R = -O(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	1.00	10	$6.3 \pm 0.3^*$	9
R = -OGer	1.00	10	$5.0 \pm 0.3^*$	28
R = -CH <sub>3</sub>	1.00	10	$4.7 \pm 0.3^*$	35
R = -I	1.00	10	$5.2 \pm 0.3^*$	28
R = -Br	1.00	10	$4.2 \pm 0.3^*$	42
R = -Cl	1.00	10	$4.7 \pm 0.2^*$	36
R = -F	1.00	10	$5.1 \pm 0.3^*$	27
Collinin	1.00	10	$3.7 \pm 0.2^*$	46
Indomethacin	0.25	10	$3.8 \pm 0.3^*$	47
Controls	--	10	$7.2 \pm 0.3^*$	--

\*p < 0.05 at Student's *t* test

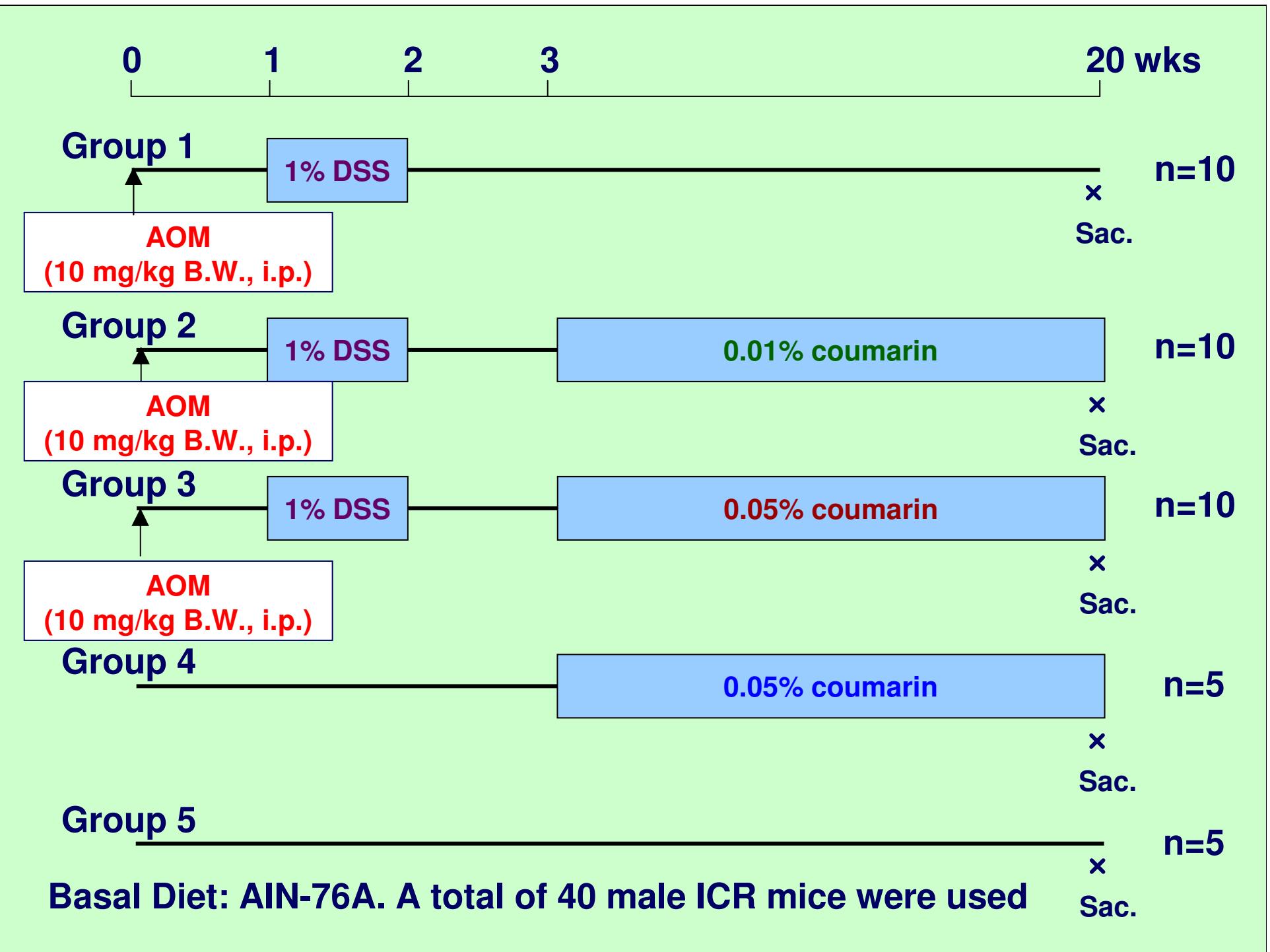
# Anticancer activity

**Chemoprevention of colon adenoma and adenocarcinoma chemically induced by administration of azoxymethane (AOM) and dextrane sodium sulfate (DSS) in the diet in mice**



$R = -H$ , auraptene

$R = -OCH_3$ , collinin

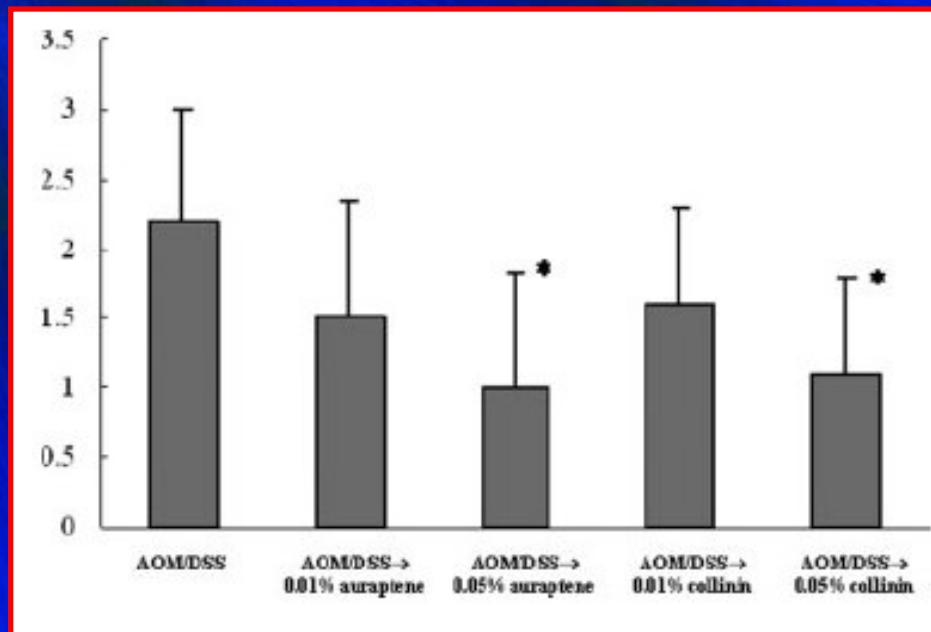


# Incidence of neoplasia after 20 weeks

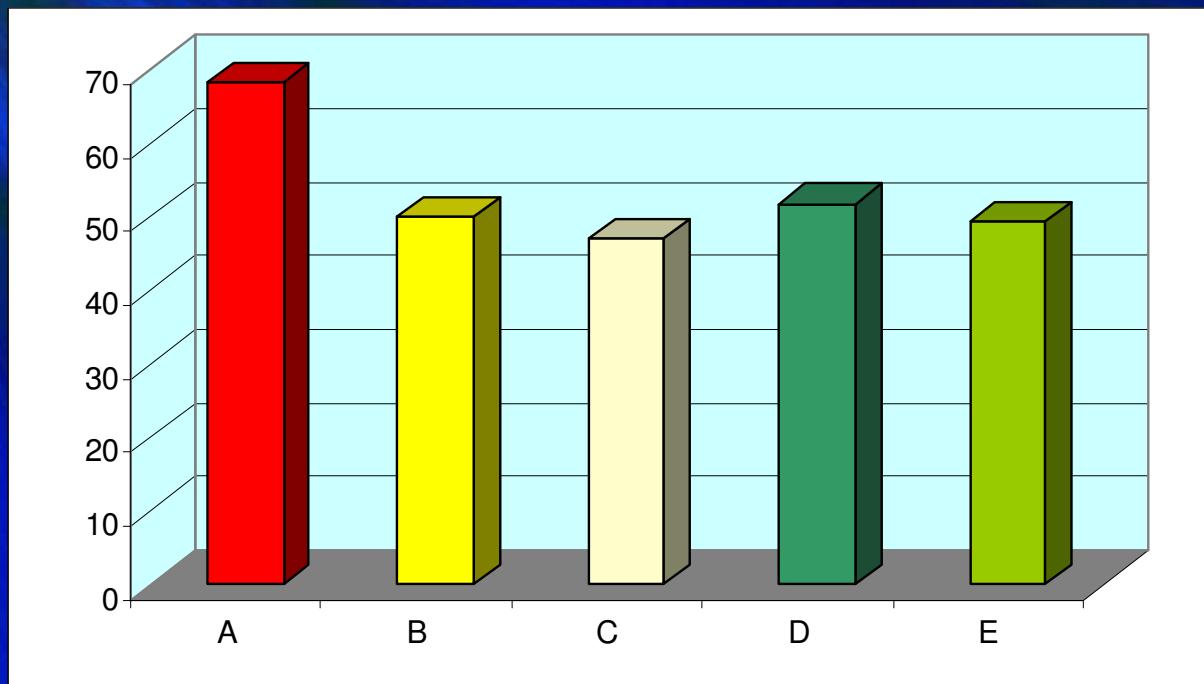
Treatment (Nr. animals)	Tot.	Adenoma	Adenocarcinoma
AOM+1% DSS (10)	10/10 (100%)	10/10 (100%)	10/10 (100%)
AOM+ 1% DSS / 0.01% auraptene (10)	8/10 (80%)	8/10 (80%)	5/10 <sup>a</sup> (50%) <sup>a</sup>
AOM+ 1% DSS / 0.05% auraptene (10)	6/10 (60%) <sup>b</sup>	6/10 (60%) <sup>b</sup>	4/10 (40%) <sup>c</sup>
AOM+ 1% DSS / 0.01% collinin (10)	7/10 <sup>b</sup> (70%)	6/10 <sup>c</sup> (60%) <sup>b</sup>	4/10 <sup>a</sup> (40%) <sup>c</sup>
AOM+ 1% DSS / 0.05% collinin (10)	6/10 (60%) <sup>b</sup>	5/10 <sup>d</sup> (50%) <sup>a</sup>	4/10 <sup>a</sup> (40%) <sup>c</sup>
AOM (5)	0/5 (0%)	0/5 (0%)	0/5 (0%)
1% DSS (5)	0/5 (0%)	0/5 (0%)	0/5 (0%)
0.05% auraptene (5)	0/5 (0%)	0/5 (0%)	0/5 (0%)
0.05% collinin (5)	0/5 (0%)	0/5 (0%)	0/5 (0%)
Controls (5)	0/5 (0%)	0/5 (0%)	0/5 (0%)

<sup>a</sup>p < 0.02 <sup>b</sup>p < 0.05 <sup>c</sup>p < 0.01 at Student's t test

## Inflammation score



# PCNA-labeling index



**A = AOM + 1% DSS**

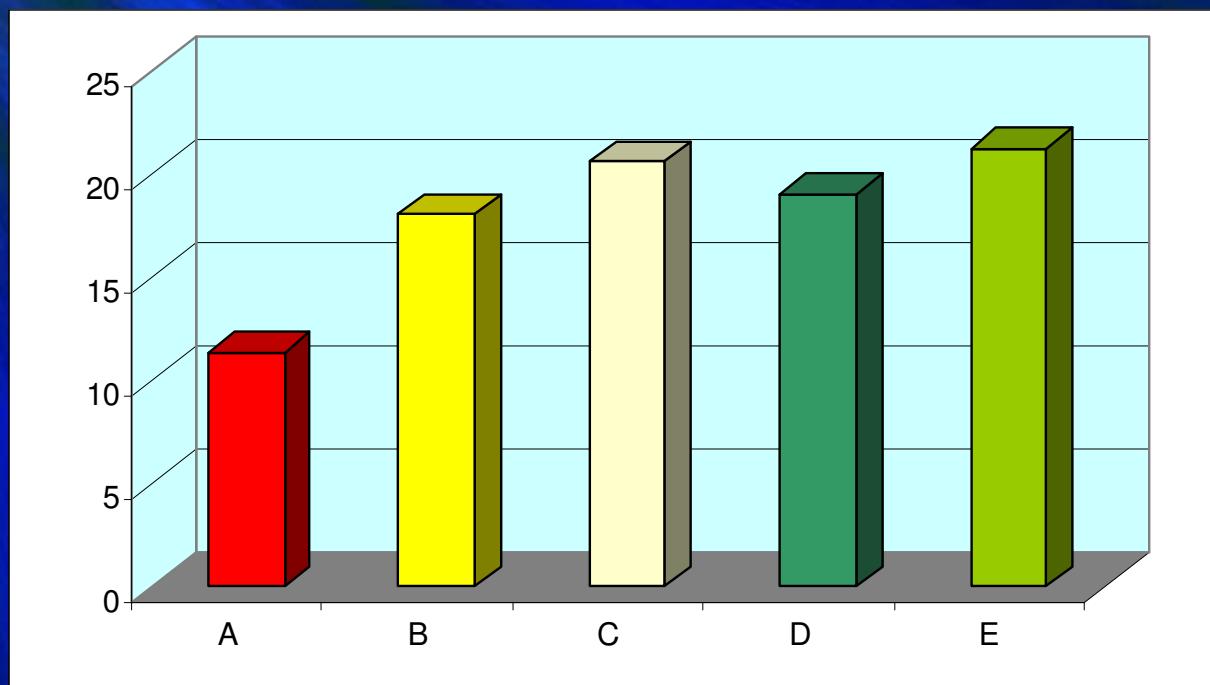
**B = AOM + 1% DSS + 0.01% auraptene**

**C = AOM + 1% DSS + 0.05% auraptene**

**D = AOM + 1% DSS + 0.01% collinin**

**E = AOM + 1% DSS + 0.05% collinin**

## Apooptotic index



**A = AOM + 1% DSS**

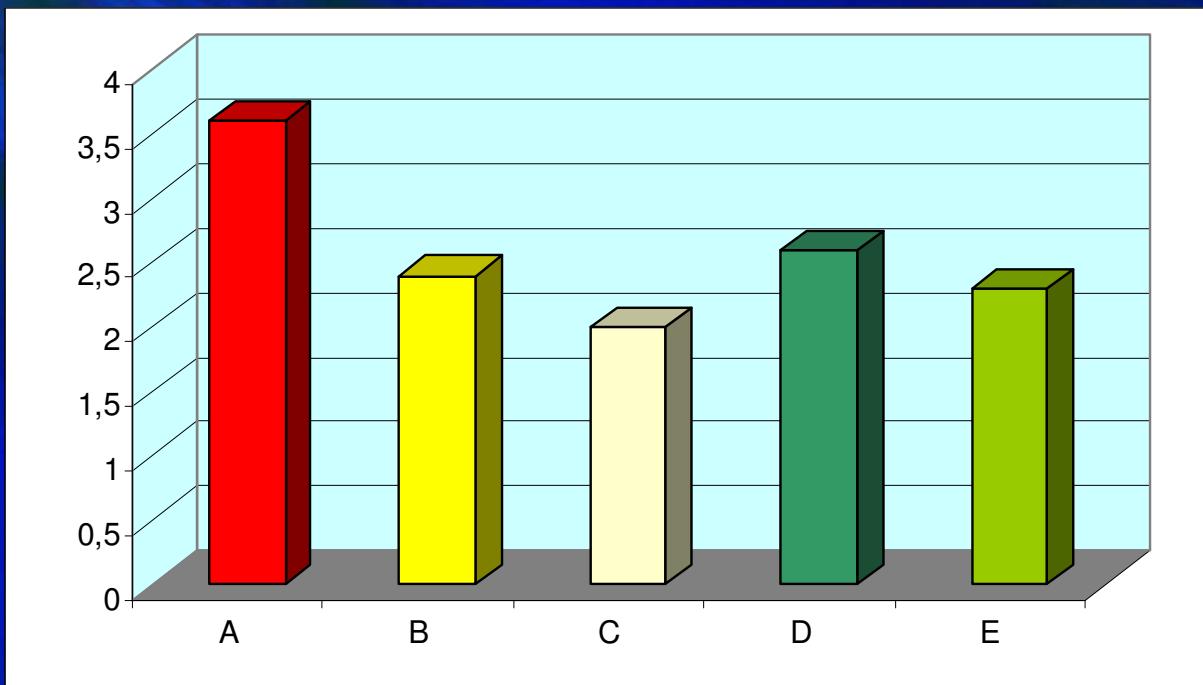
**B = AOM + 1% DSS + 0.01% auraptene**

**C = AOM + 1% DSS + 0.05% auraptene**

**D = AOM + 1% DSS + 0.01% collinin**

**E = AOM + 1% DSS + 0.05% collinin**

## COX-2 expression



**A = AOM + 1% DSS**

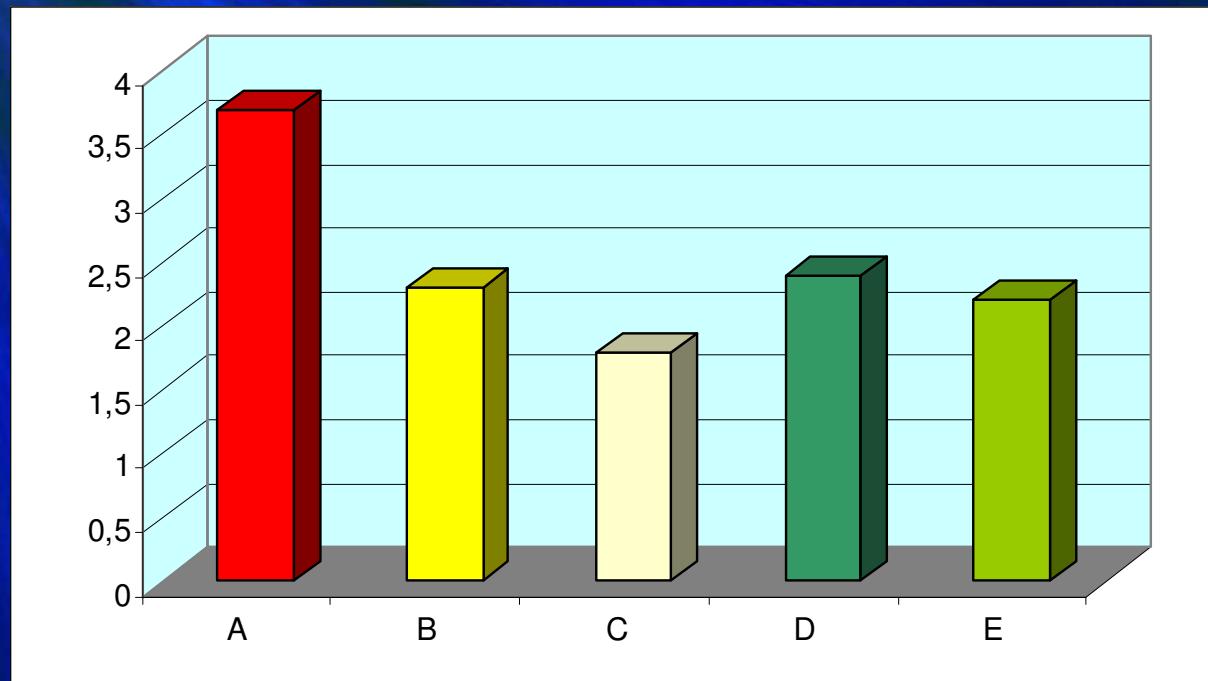
**B = AOM + 1% DSS + 0.01% auraptene**

**C = AOM + 1% DSS + 0.05% auraptene**

**D = AOM + 1% DSS + 0.01% collinin**

**E = AOM + 1% DSS + 0.05% collinin**

## **NOS expression**



**A = AOM + 1% DSS**

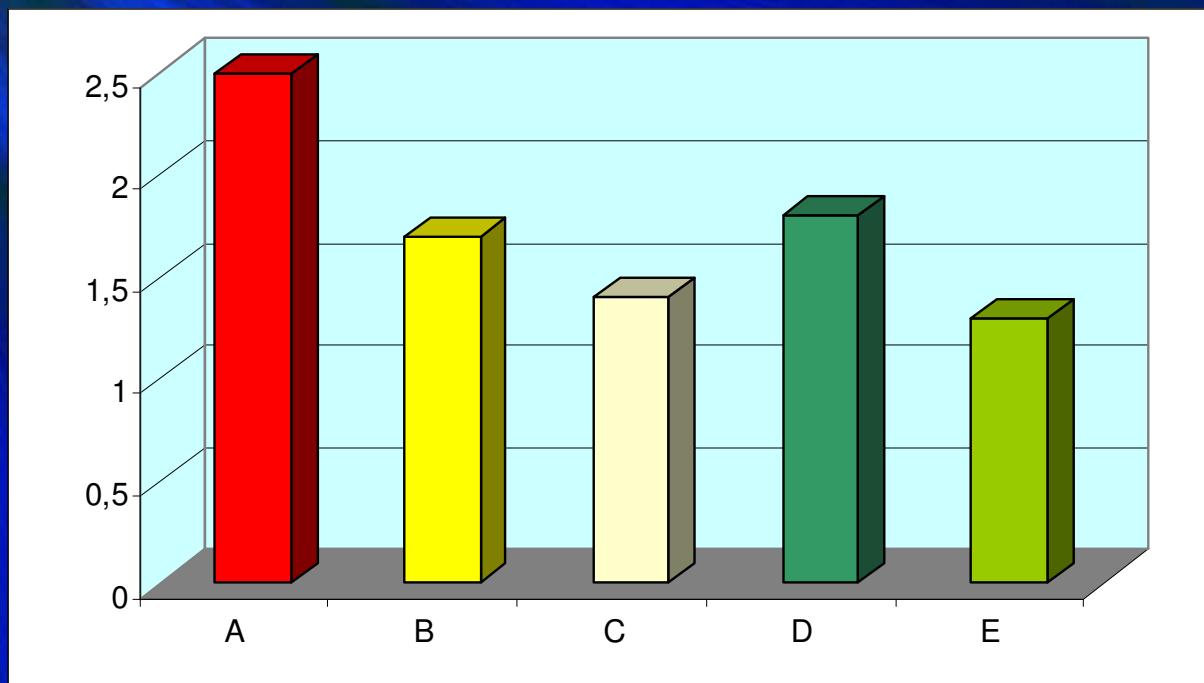
**B = AOM + 1% DSS + 0.01% auraptene**

**C = AOM + 1% DSS + 0.05% auraptene**

**D = AOM + 1% DSS + 0.01% collinin**

**E = AOM + 1% DSS + 0.05% collinin**

# Nitrotyrosine



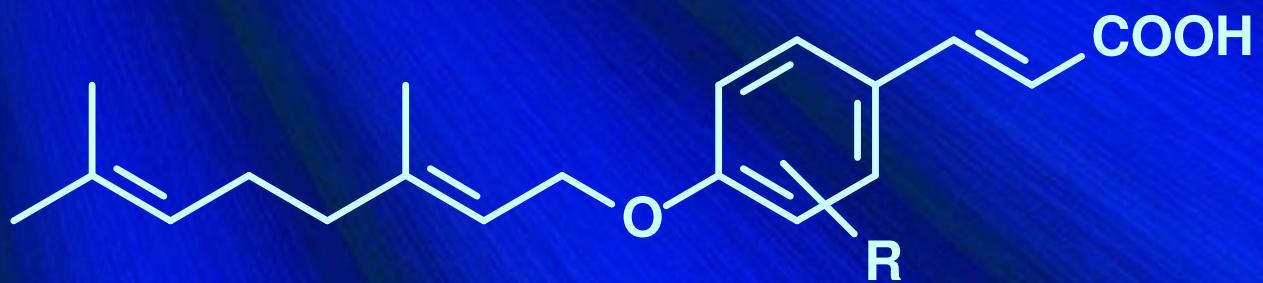
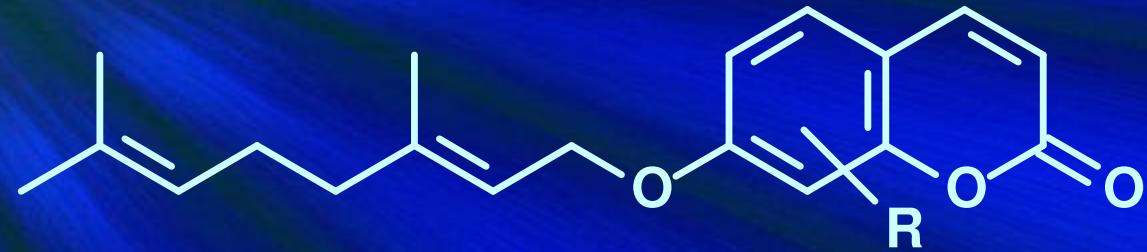
**A = AOM + 1% DSS**

**B = AOM + 1% DSS + 0.01% auraptene**

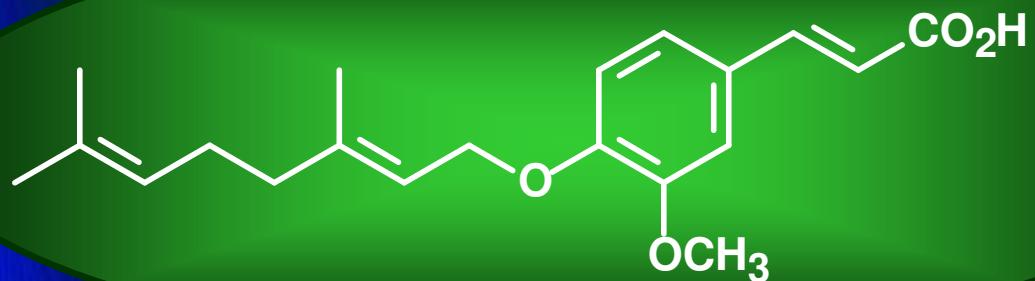
**C = AOM + 1% DSS + 0.05% auraptene**

**D = AOM + 1% DSS + 0.01% collinin**

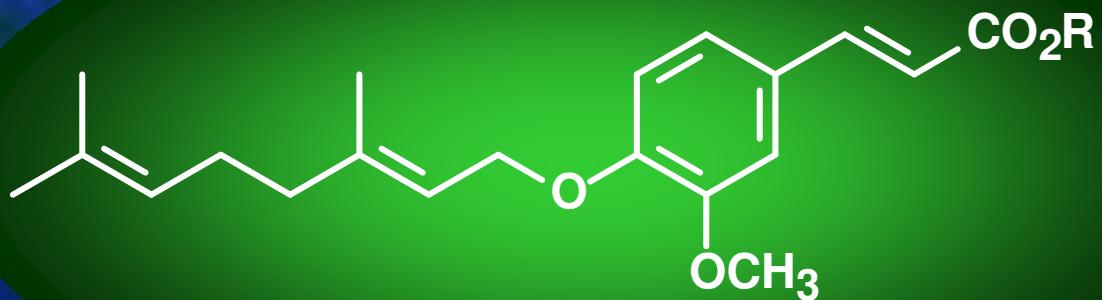
**E = AOM + 1% DSS + 0.05% collinin**



*Acronychia baueri* Schott  
Sin. *Bauerella simplicifolia*  
*Sarcomelicope simplicifolia*  
(Rutaceae)

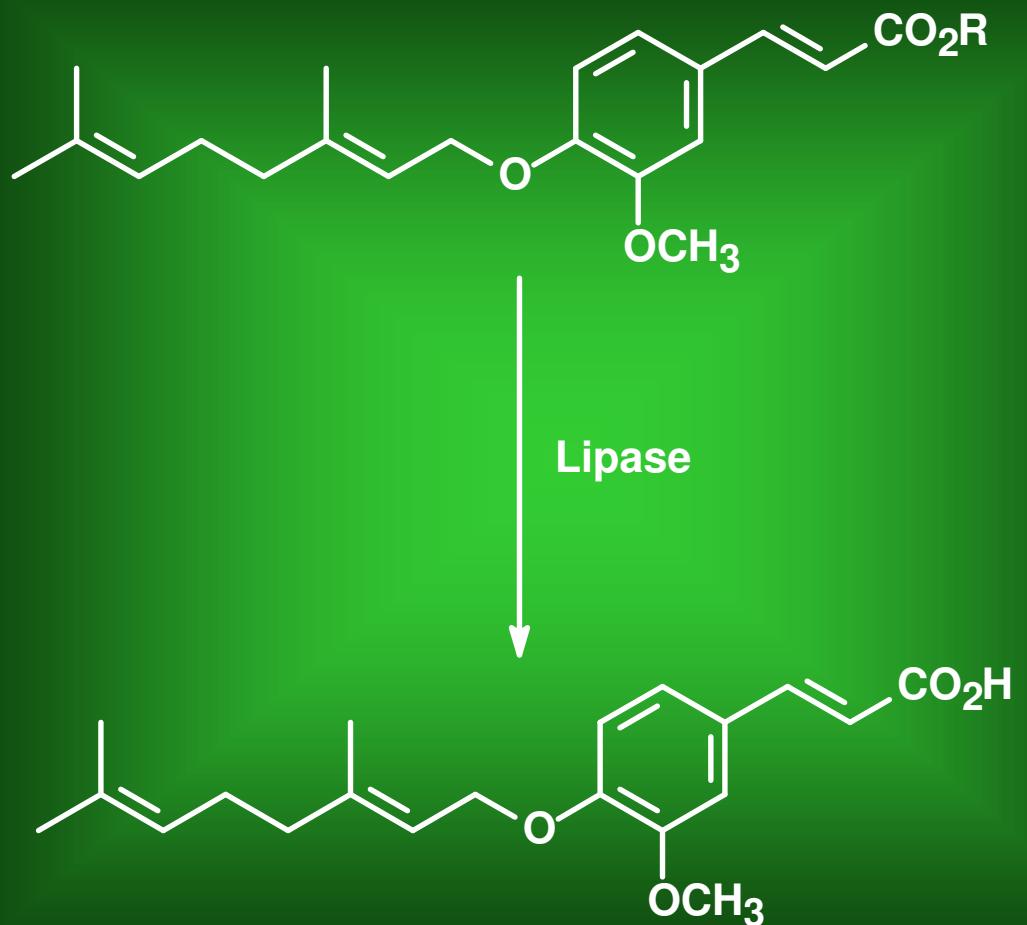


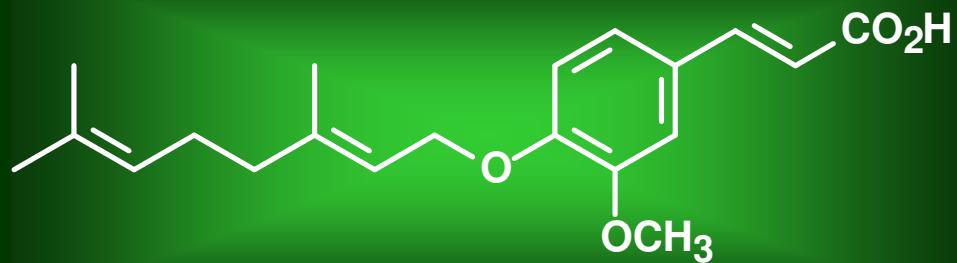
Prager, R.H.; Thregold, H.M. *Aust. J. Chem.* 1966, 19, 451



Tongue and colon cancer chemopreventive agent

NOS and COX-2 inhibitor





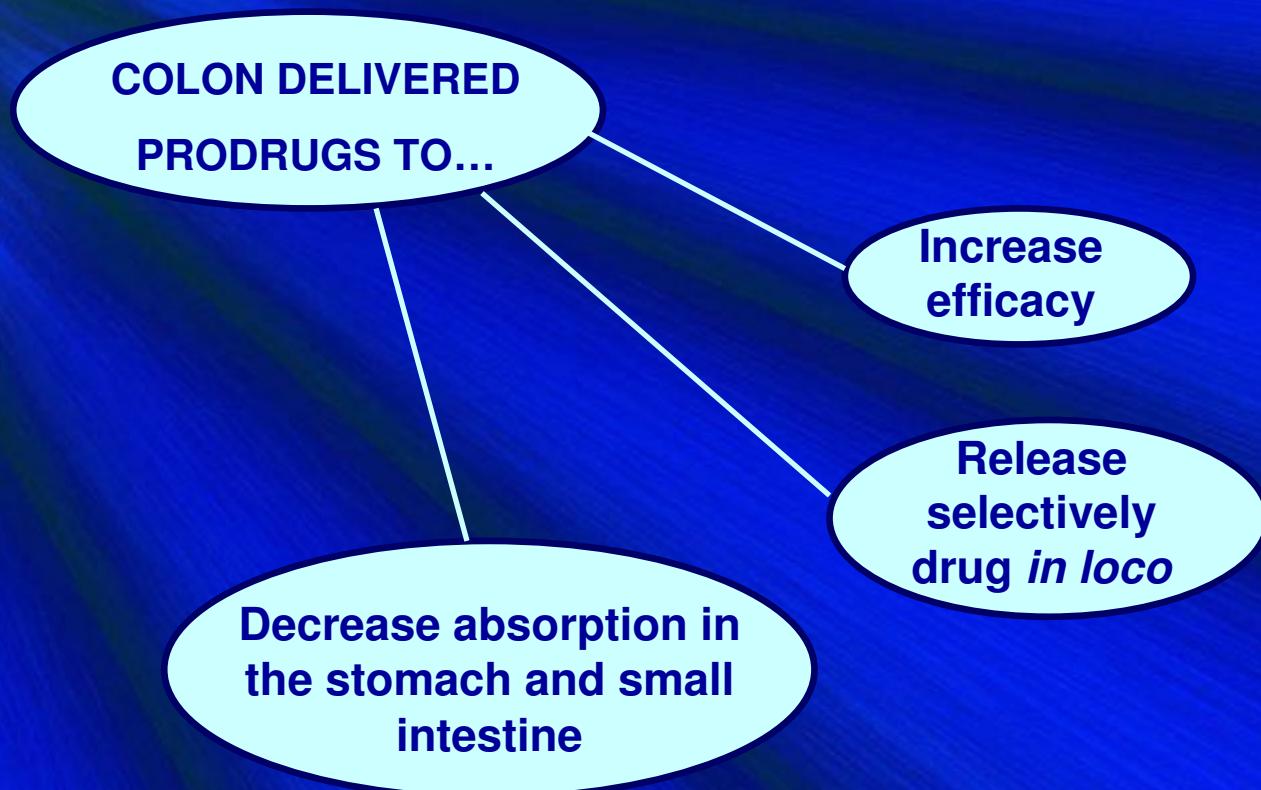
As colon cancer  
chemopreventive agent  
*per os...*

High lipophilicity

Partial absorption in the upper portion of  
gastrointestinal tract

**LOWER BIOAVAILABILITY**

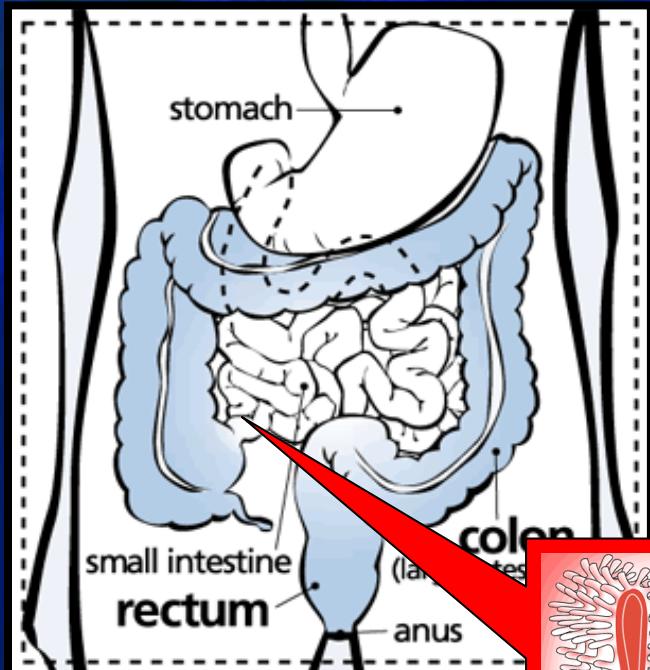
## Novel approaches for the treatment of bowel diseases...



# HIGHER BIOAVAILABILITY

# Which kind of prodrug?

Enzymatic cleavage of prodrugs containing an oligopeptide chain

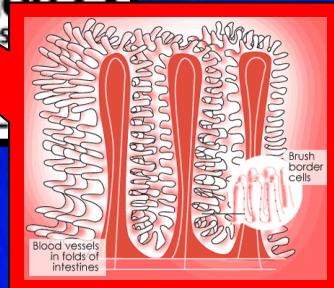


AMINO- AND CARBOXYPEPTIDASES

ACE

Intestinal “Angiotensin Converting Enzyme”  
(dipeptidyl carboxypeptidase, E.C. 3.4.15.1)

X-Ala-Pro-COOH

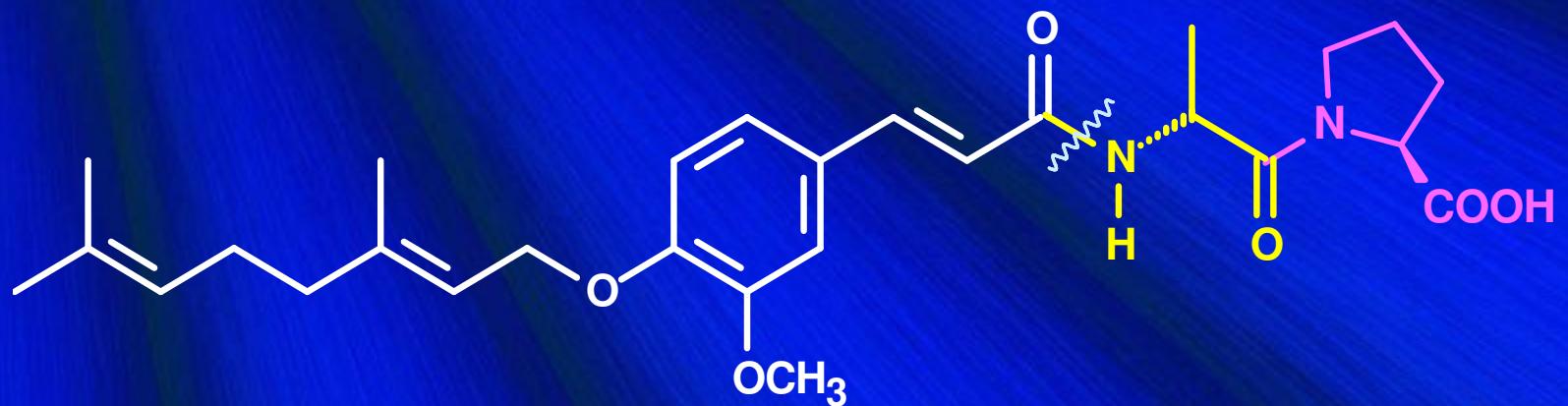


Yoshioka, M. ; Erickson, R. H. ; Kim, Y.S. K. *J. Clin. Invest.* 1988, 81, 174

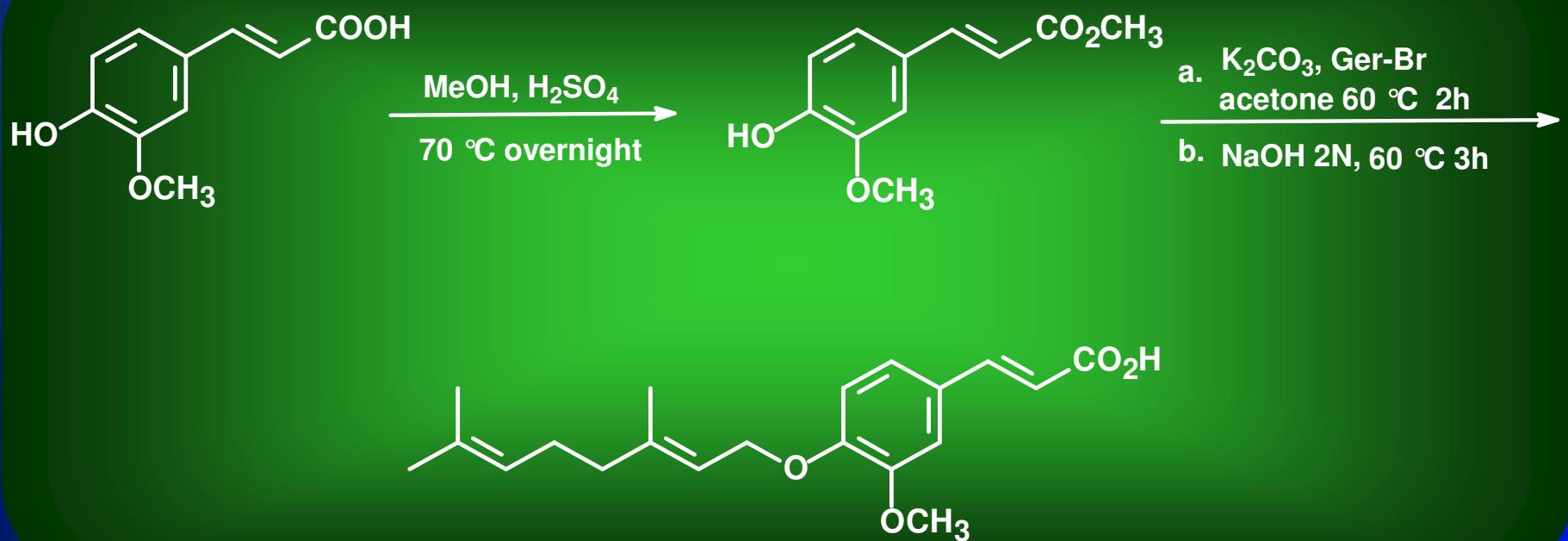
ACE

Angiotensin Converting Enzyme

X<sup>w</sup>Ala—Pro—COOH

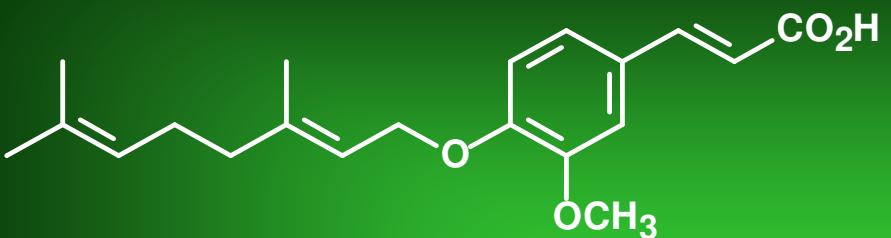


## Step 1: synthesis of the free acid



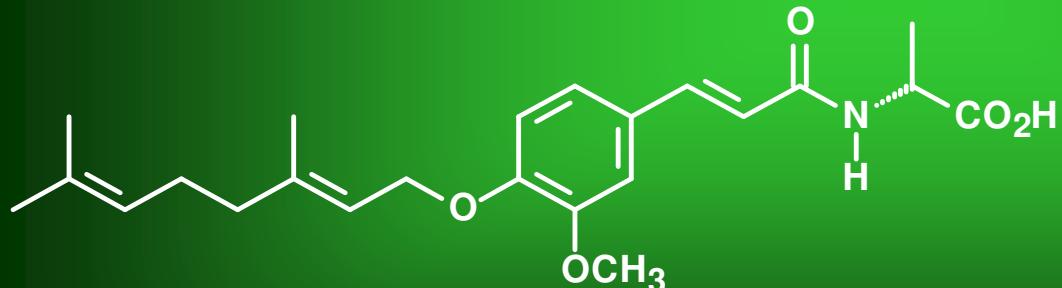
98% overall yield

## Step 2: conjugation of the free acid to Ala-Pro



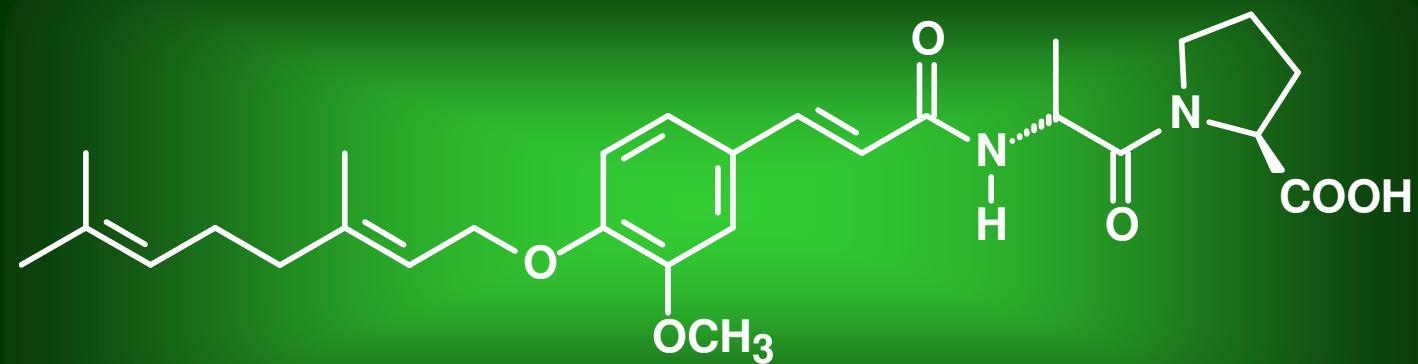
c. L-AlaOMe.HCl, DCC, Et<sub>3</sub>N  
Et<sub>2</sub>O, t.a., 6h

d. NaOH 0.5N,  
EtOH/H<sub>2</sub>O 1:1 30min



e. L-ProOMe.HCl, DCC, Et<sub>3</sub>N  
Et<sub>2</sub>O, t.a., 6h

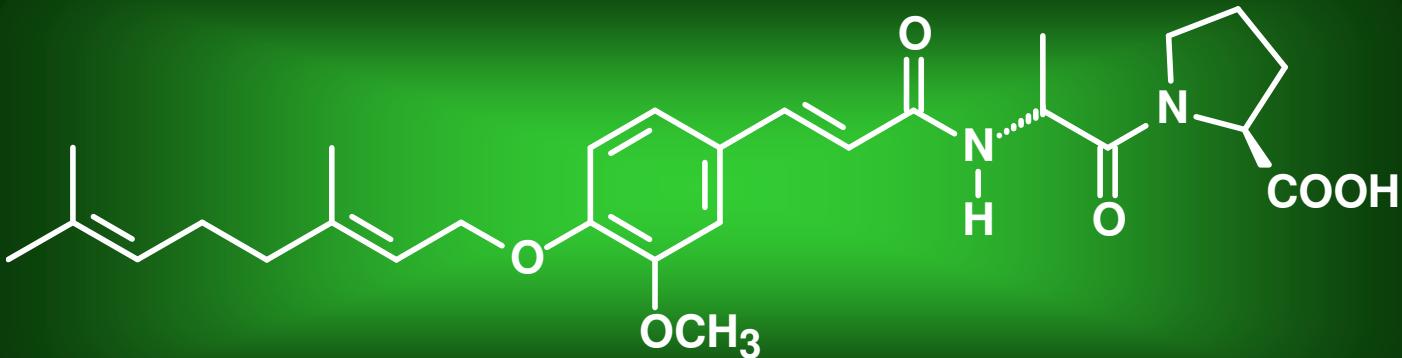
f. NaOH 0.5N,  
EtOH/H<sub>2</sub>O 1:1 30min



**56.6% overall yield**

Curini, M.; Epifano, F.; Genovese, S. *Bioorg. Med. Chem. Lett.* 2005, 15, 5049

## Step 3: chemical and enzymatic stability



pH 0.5 ÷ 9.5, 37 °C, 4h

No hydrolysis

Isolated ACE, 37 °C, 2h

> 99% hydrolysis

Landon, M. ; *Methods Enzymol.* 1977, 47, 145

Yoshioka, M. ; Erickson, R. H. ; Kim, Y.S. K. *J. Clin. Invest.* 1988, 81, 174

# Incidence and multiplicity of colonic tumors

Treatment (nr. animals)	Incidence of colonic tumors			Multiplicity (tumors/mouse)		
	Total	AD	ADC	Total	AD	ADC
AOM + DSS (20)	20 (100%)	19 (95%)	19 (95%)	5.60±4.81 <sup>a</sup>	2.50±2.37	3.10±3.06
AOM + DSS + 0.01% GAP (15)	10 (67%)	8 (53%)	9 (60%)	2.33±2.12 <sup>b</sup>	1.20±1.27	1.13±1.13 <sup>b</sup>
AOM + DSS + 0.05% GAP (15)	10 (67%)	8 (53%)	8 (53%)	2.00±2.10 <sup>b</sup>	1.20±1.37	0.80±1.08 <sup>c</sup>
0.05% GAP (5)	0 (0%)	0 (0%)	0 (0%)	0.00±0.00	0.00±0.00	0.00±0.00
None (5)	0 (0%)	0 (0%)	0 (0%)	0.00±0.00	0.00±0.00	0.00±0.00

a Mean ± SD. AD= adenoma and ADC=adenocarcinoma.

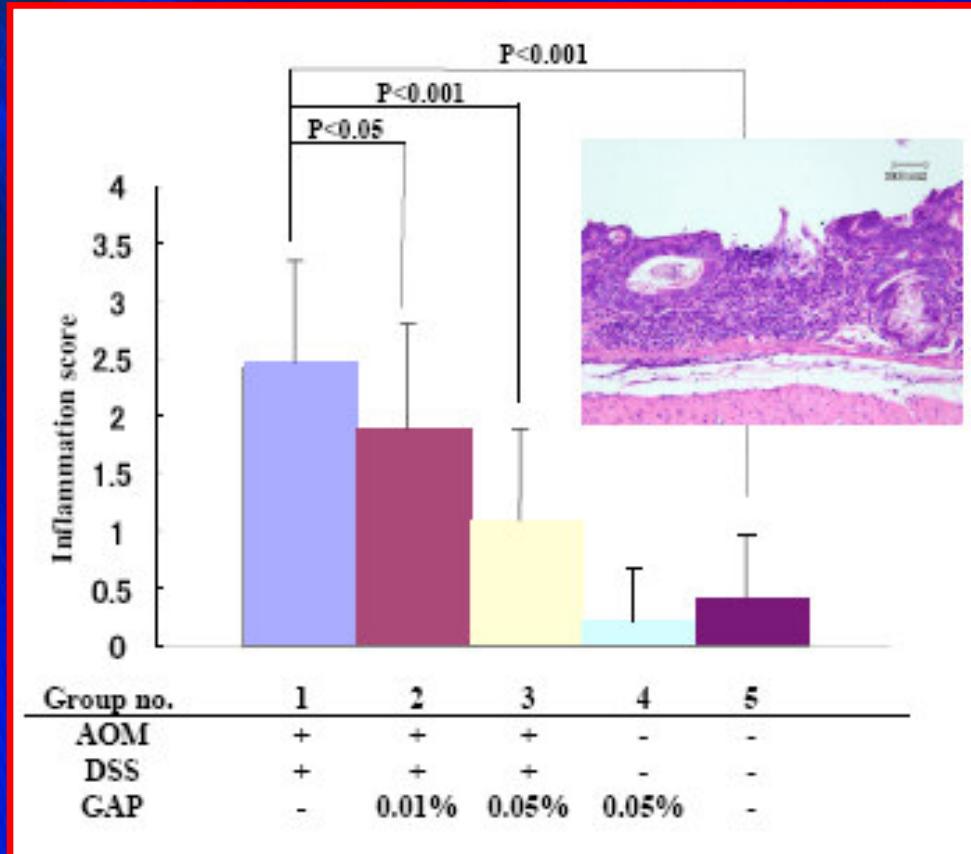
b,c Significantly different from “AOM+DSS” group (b P < 0.05 and c P < 0.01)

Linear regression:

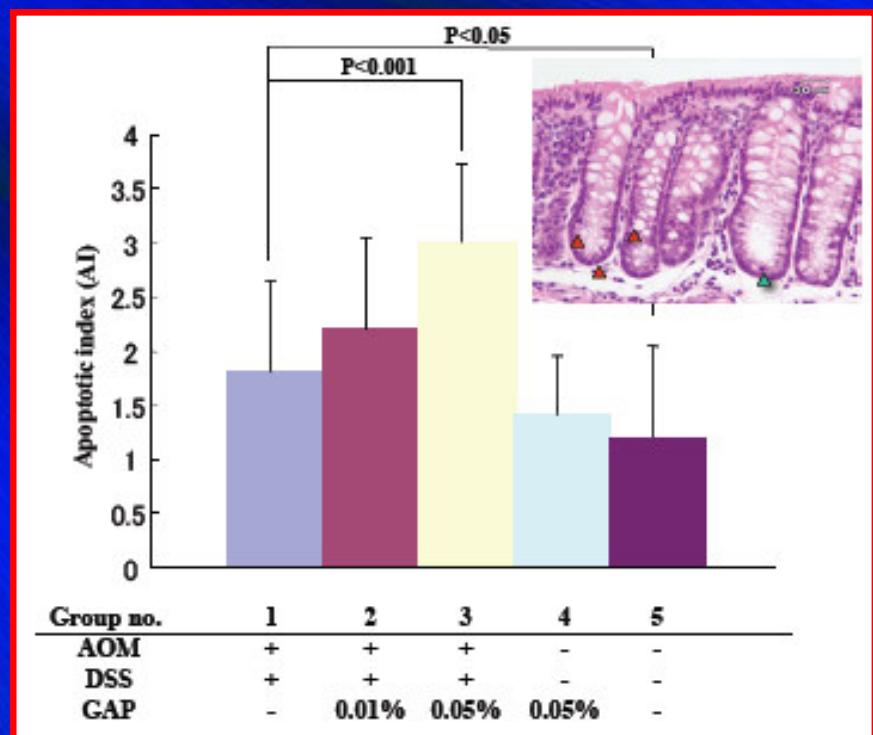
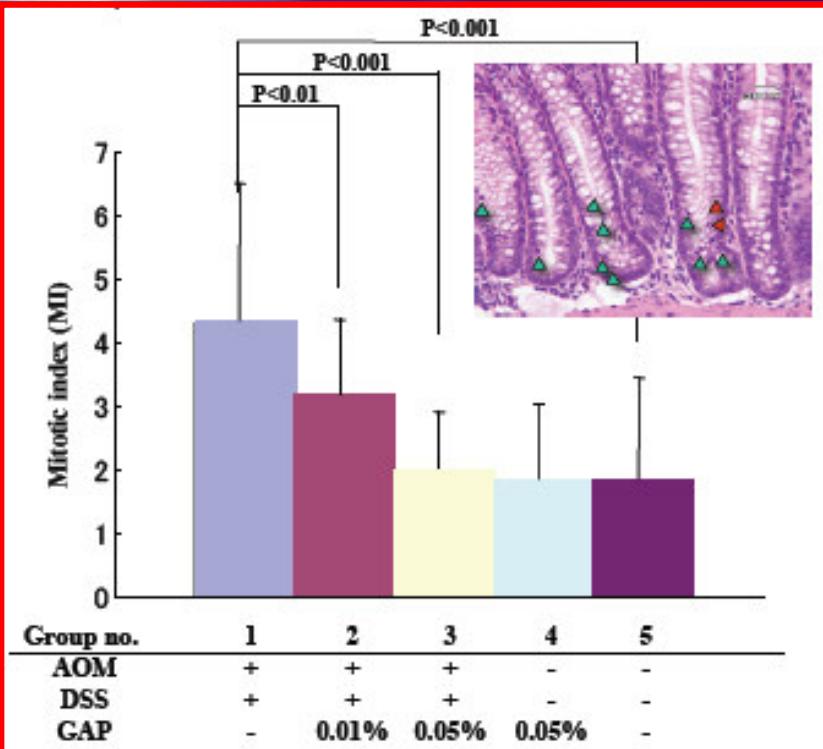
Multiplicity of total tumors: Correlation coefficient (r) = -0.72, y = -53.79x+4.39, P = 0.0027

Multiplicity of adenocarcinomas: Correlation coefficient (r) =-0.75, y = -35.21x+2.38, P = 0.0039

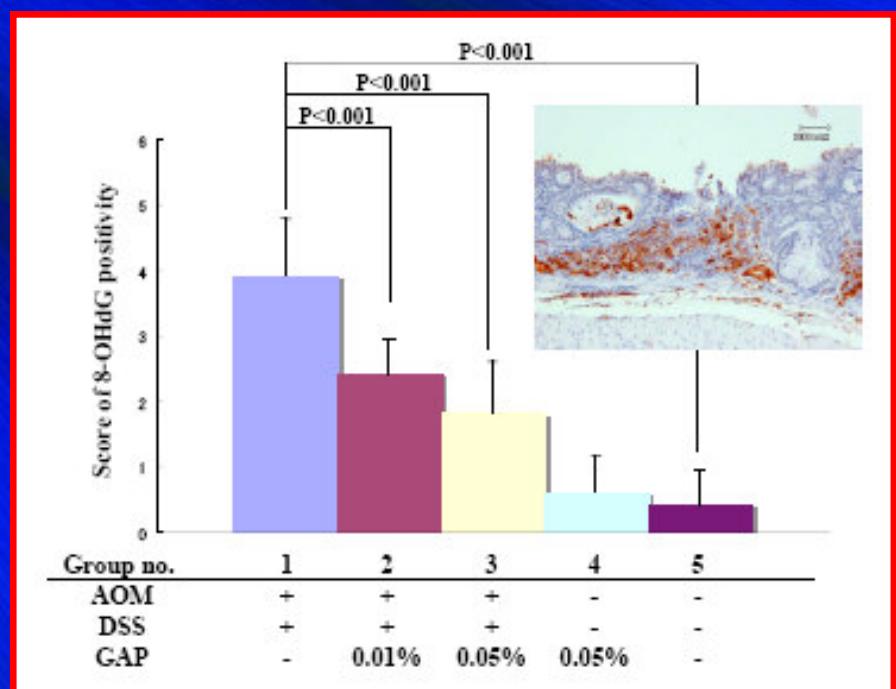
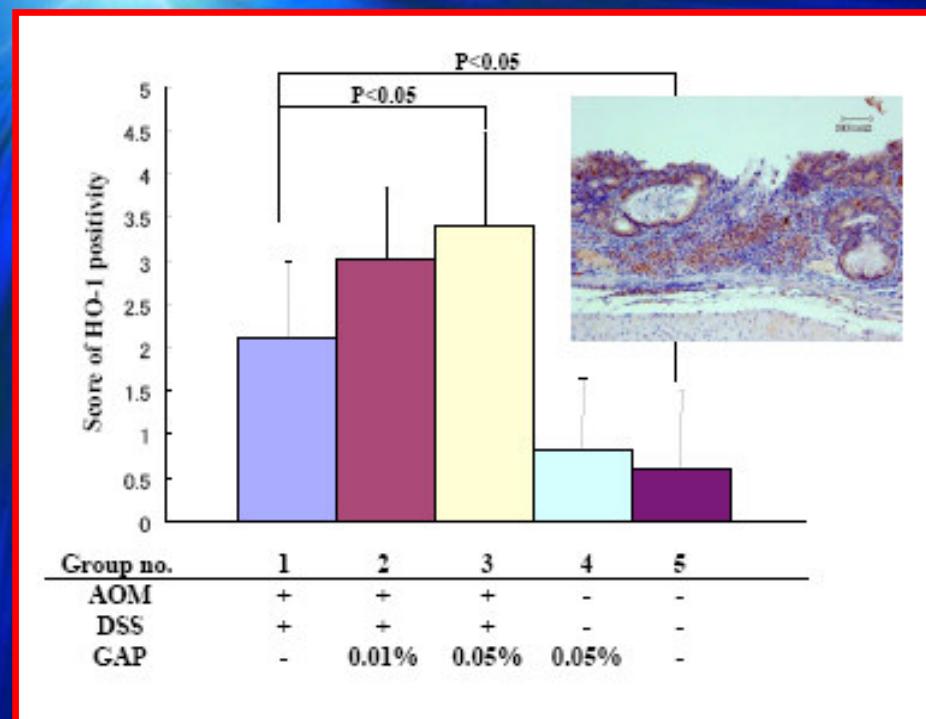
# Inflammation score



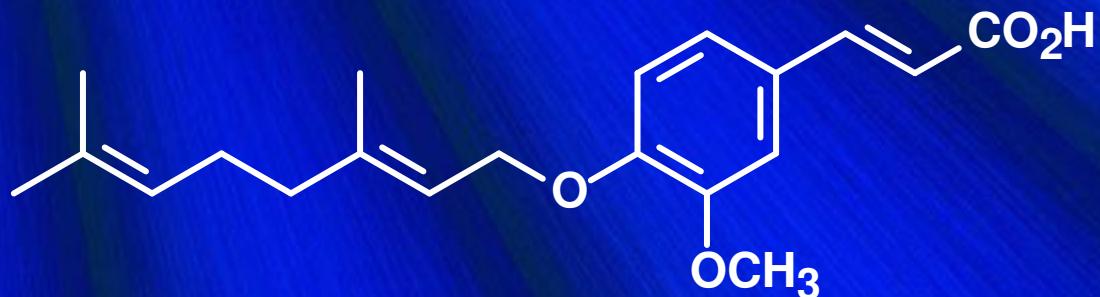
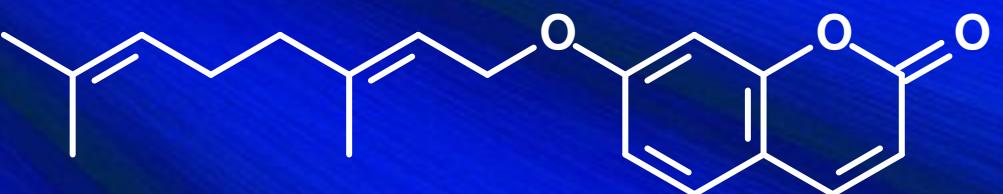
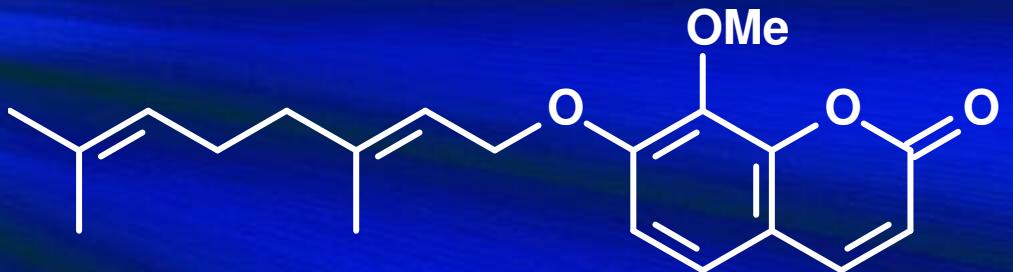
## Cell proliferation indices



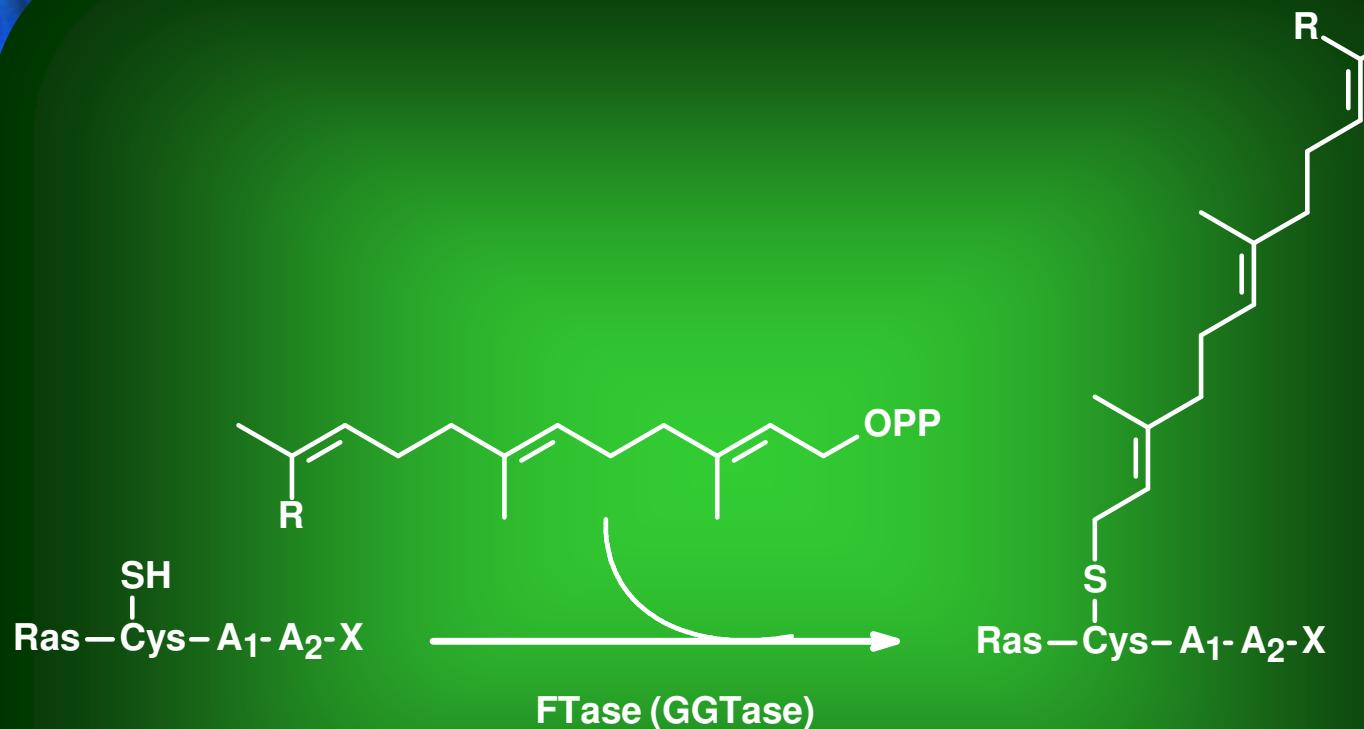
## Oxidative stress indices



Trying to  
understand  
the  
mechanism  
of action...



# PRENYLTRANSFERASES



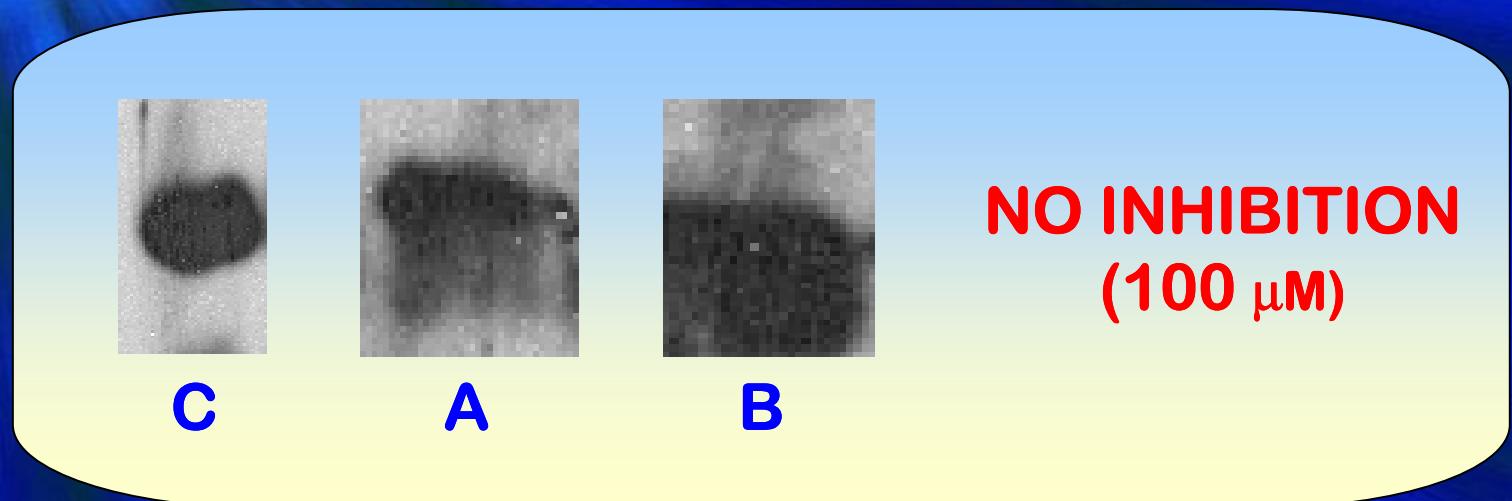
$R = H$  (FDP)

$R =$  isopentenyl (GGDP)

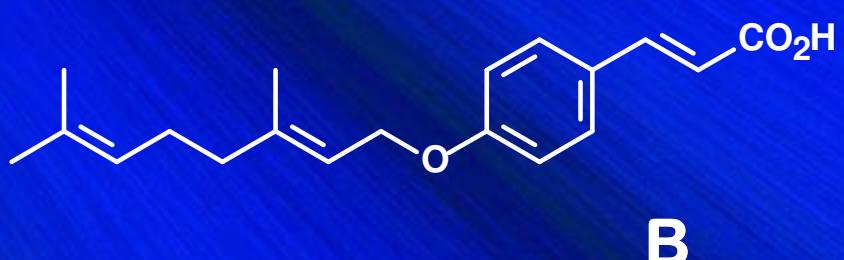
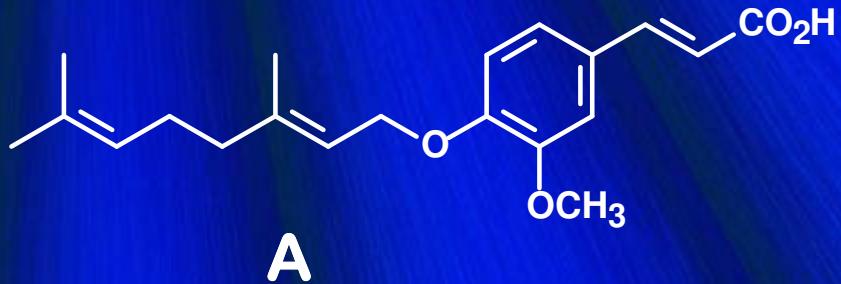
# PRENYLTRANSFERASES INHIBITION *IN VITRO*

Compound	% inhibition (100μM)	
	FTase	GGTase
1	13.4 ± 6.4	78.6 ± 12.8
2	12.7 ± 23	72.4 ± 9.4
3	5.5 ± 0.6	3.0 ± 13.4
4	7.4 ± 22.1	7.5 ± 21.5
5	N.S.	N.S.
6	N.S.	N.S.

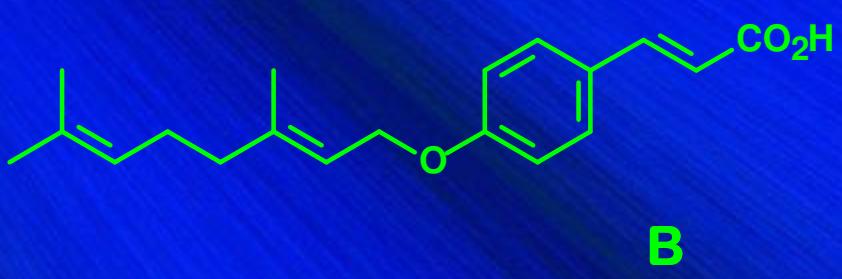
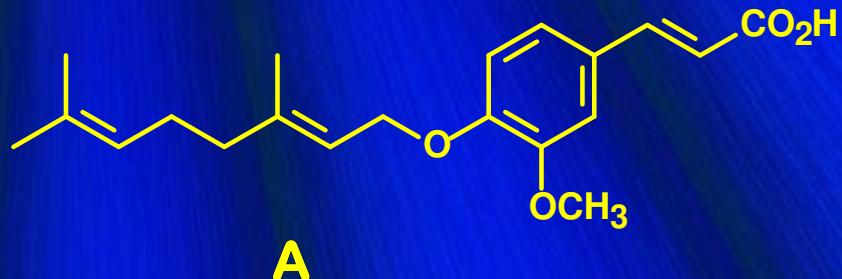
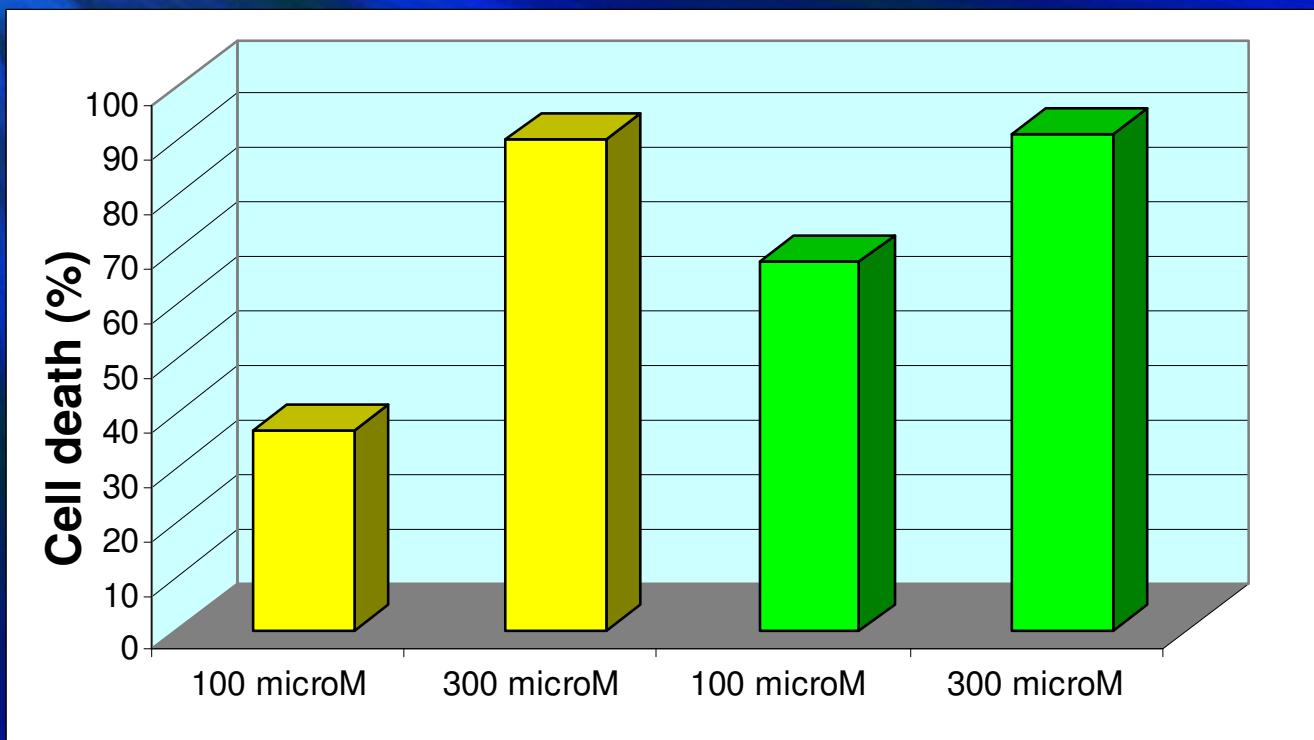
## GGTase inhibition in HRAS / 3T3 cells



C = controls

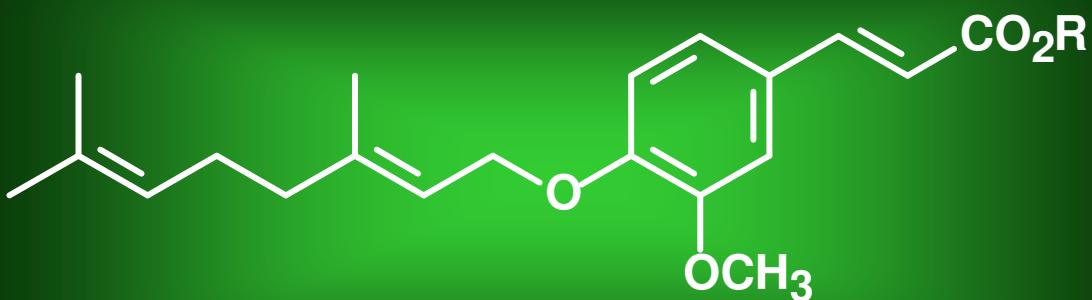


# Effects on proliferation in HRAS / 3T3 cells

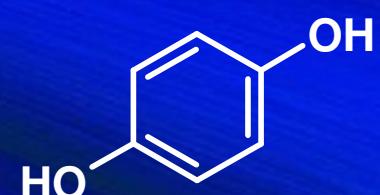
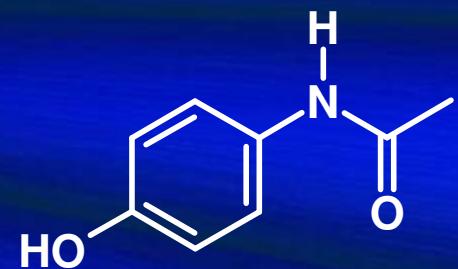
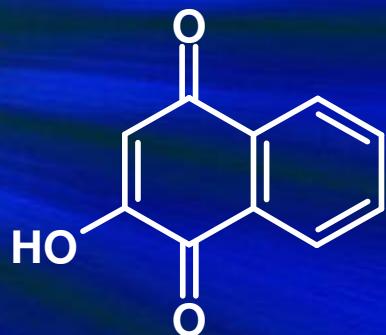
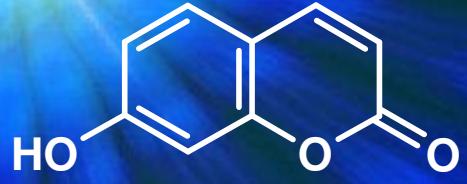




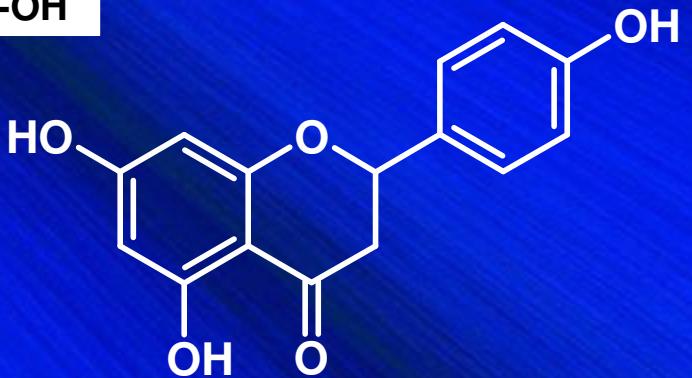
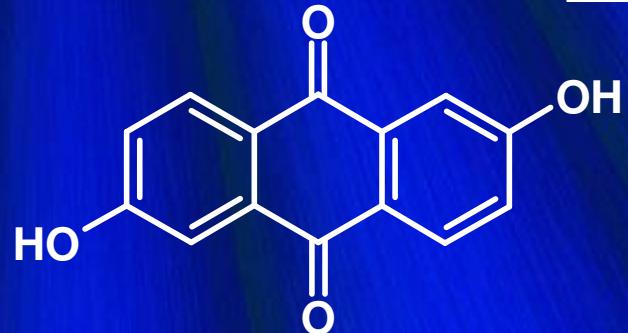
## Synthesis of esters

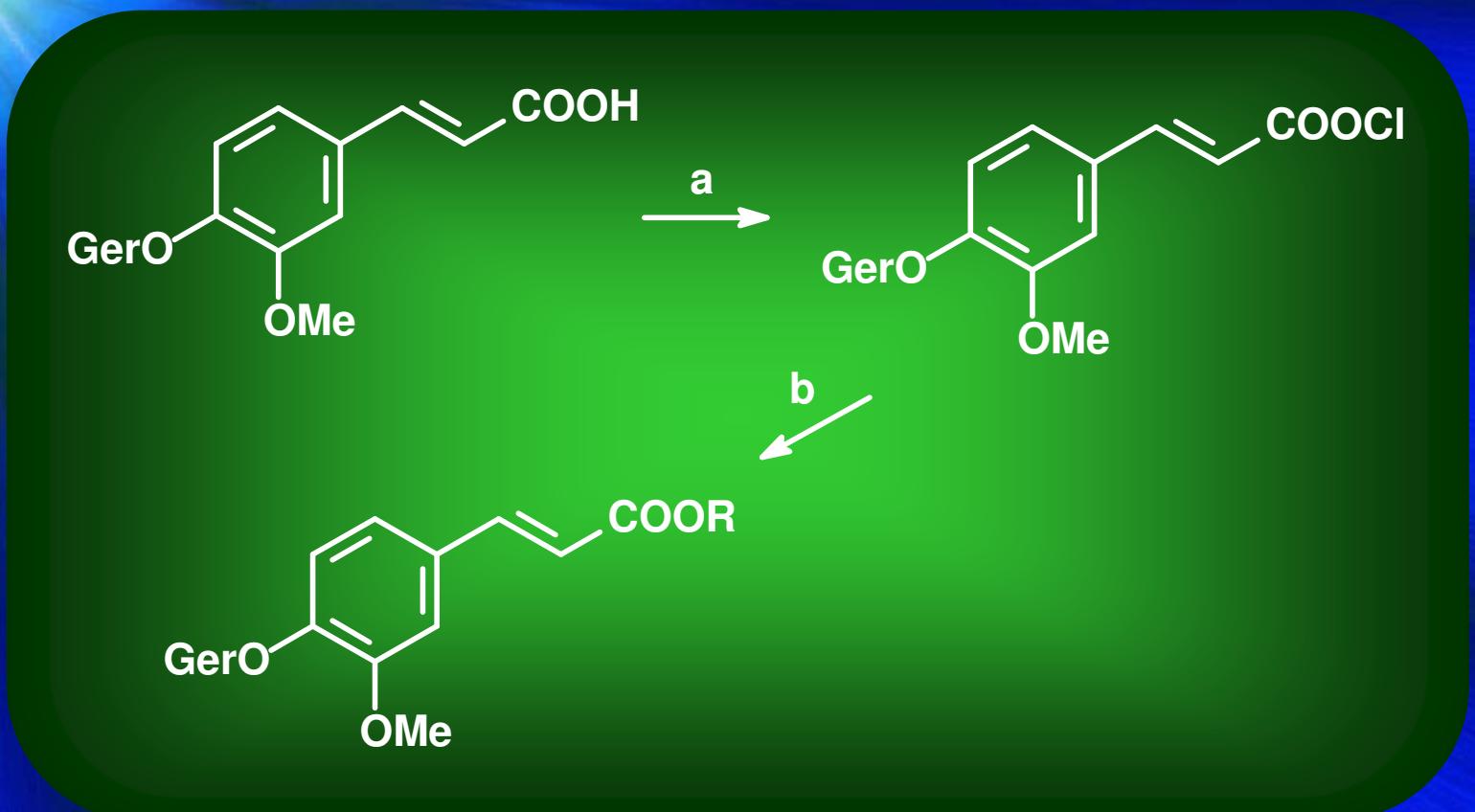


**R = natural or semi-synthetic  
pharmacologically active compounds**



R1 = -CH=CH-COOH; R2 = -OMe  
R1 = -COOH; R2 = -OMe  
R1 = -H; R2 = -OMe  
R1 = -CH=CH-COOH; R2 = -OH

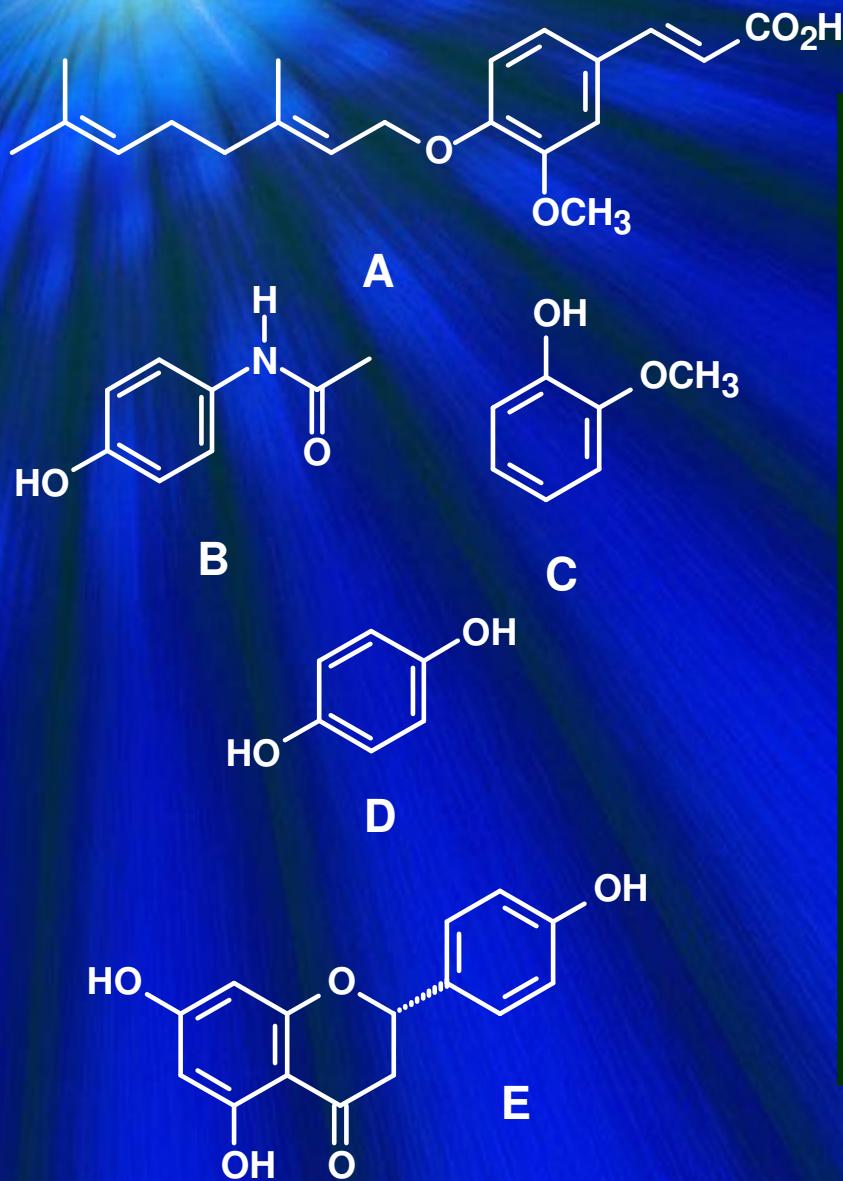




**a**  $(COCl)_2$ , dry  $Et_2O$ , r.t. 5 min.; **b** ROH ( $ArOH$ ),  $Et_3N$ , dry  $Et_2O$ , r.t. 40 min.

31 – 95 % yield

# Inhibition of the Croton oil-induced ear oedema in mice

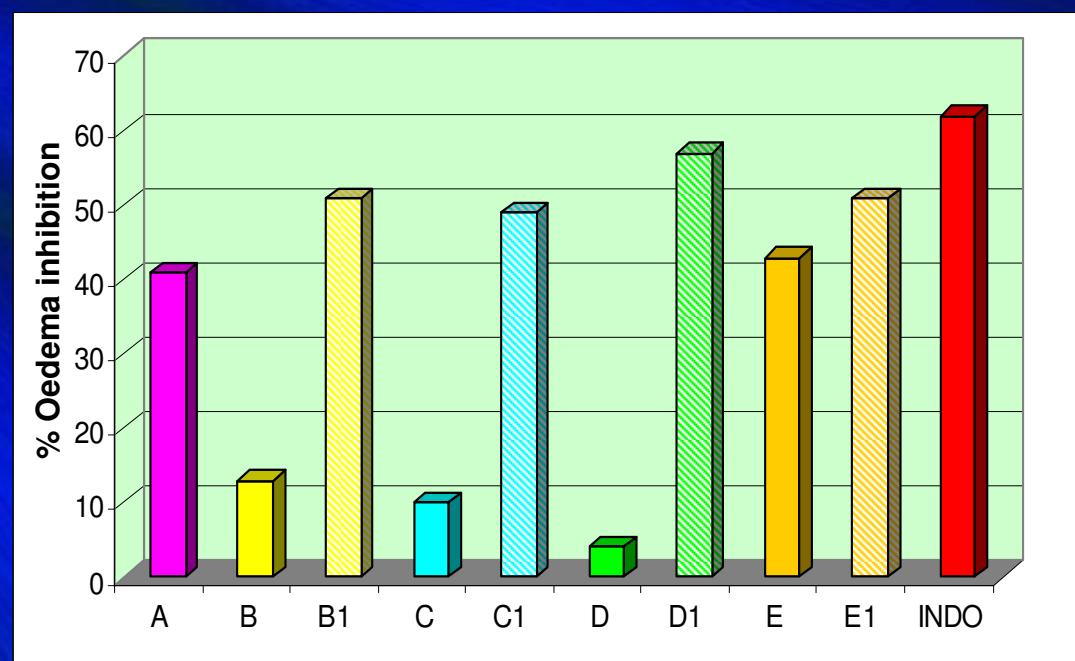
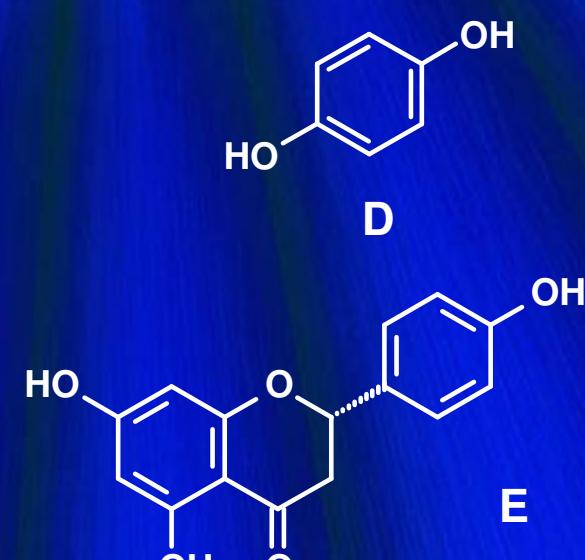
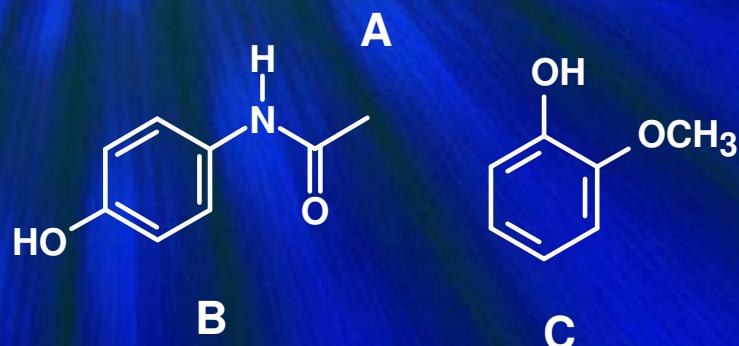
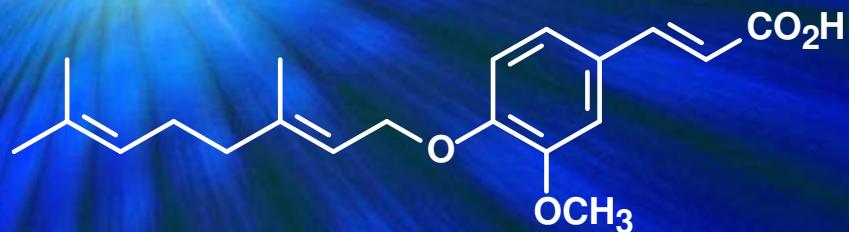


Compound	N° an.	Oedema (mg) Mean ± S.E.	% Red.
Controls	30	6.9 ± 0.3	--
<b>A</b>	30	4.1 ± 0.2*	41
<b>B</b>	10	6.0 ± 0.4	13
<b>B1</b>	10	3.4 ± 0.4* <sup>o</sup>	51
<b>C</b>	10	6.2 ± 0.4	10
<b>C1</b>	10	3.5 ± 0.3* <sup>o</sup>	49
<b>D</b>	10	6.6 ± 0.4	4
<b>D1</b>	10	3.0 ± 0.3* <sup>o</sup>	57
<b>E</b>	10	3.9 ± 0.3*	43
<b>E1</b>	10	3.4 ± 0.3*	51
<b>Indomethacin</b>	10	2.6 ± 0.2*	62

\*p<0.05 at the analysis of variance, as compared with controls

<sup>o</sup>p<0.05 at the analysis of variance, as compared with compound A

# Inhibition of the Croton oil-induced ear oedema in mice



Epifano, F.; Genovese, S.; Sosa, S.; Tubaro, A.; Curini, M. *Bioorg. Med. Chem. Lett.* 2007, 17, 5709-14

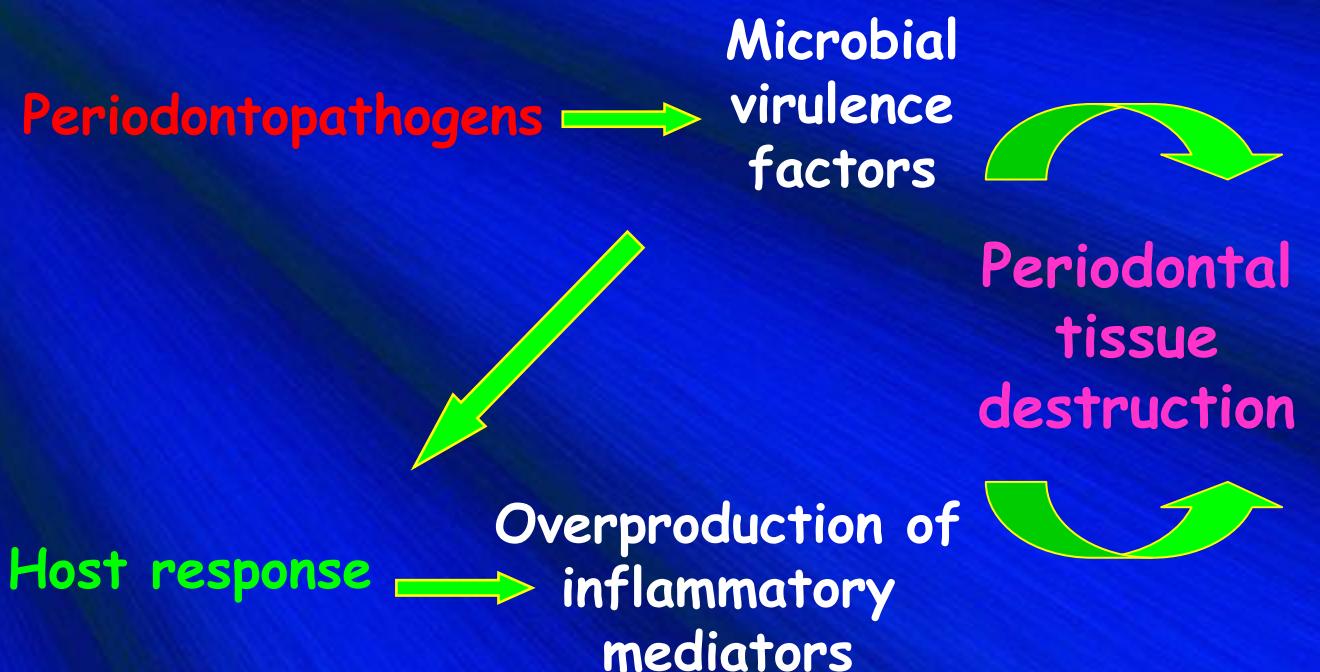
# Antibacterial activity: inhibition of biofilm formation by *Porphyromonas gingivalis* and *Streptococcus mutans*

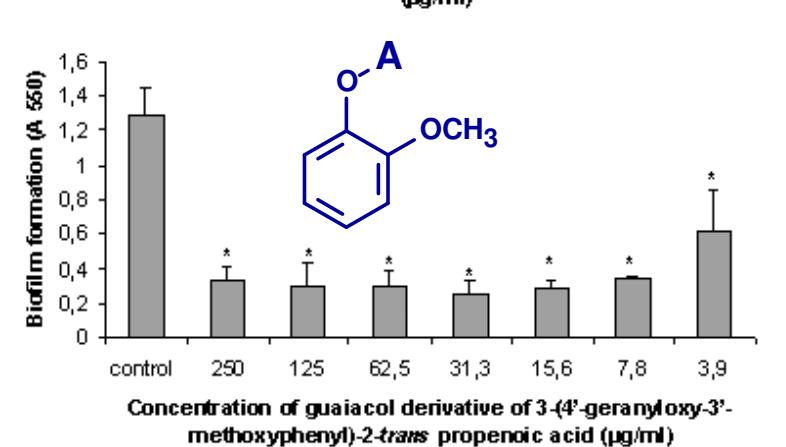
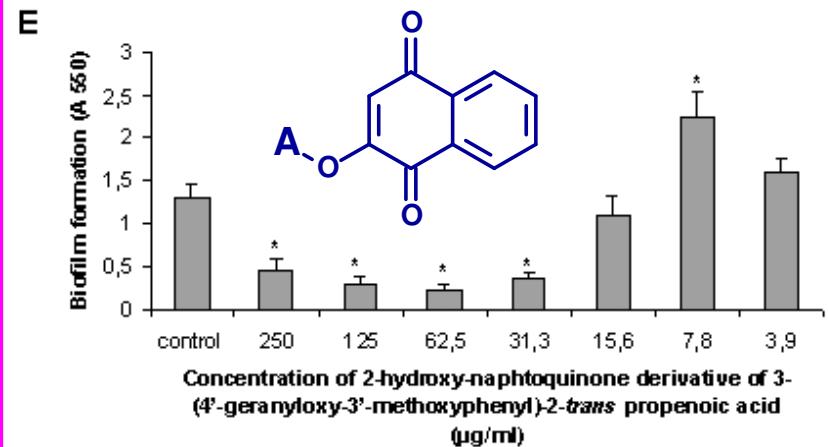
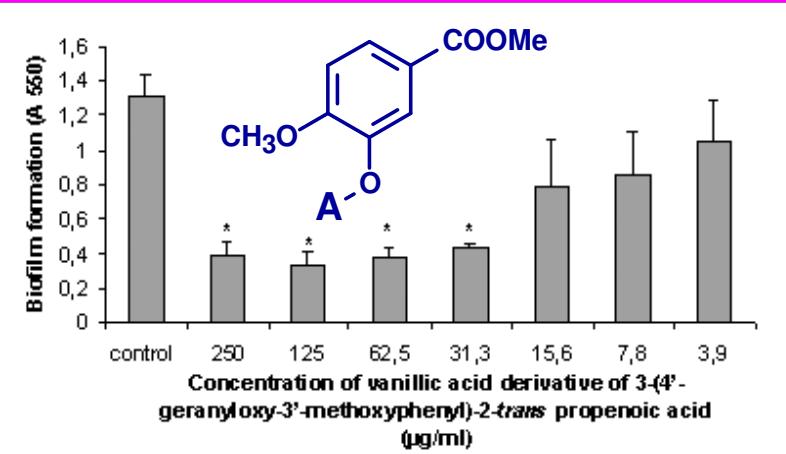
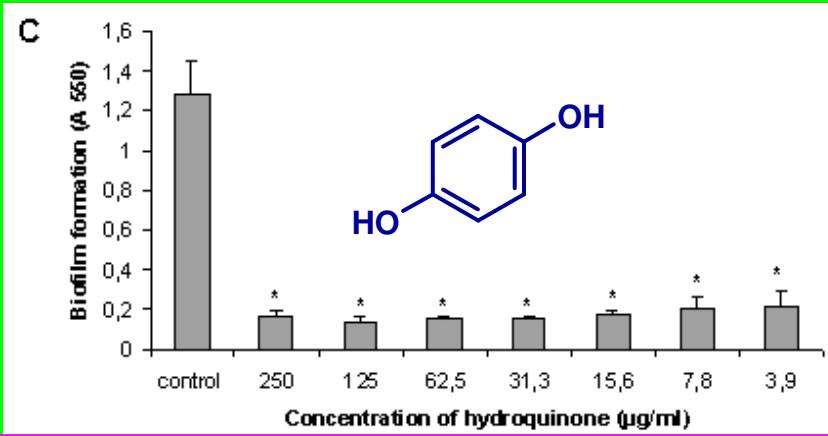
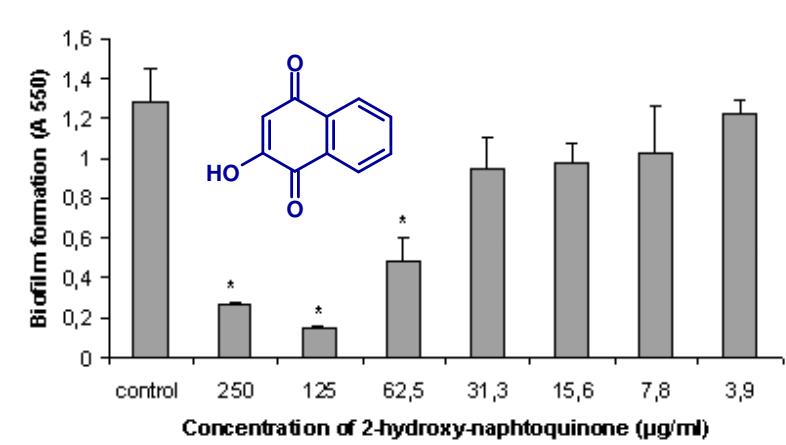
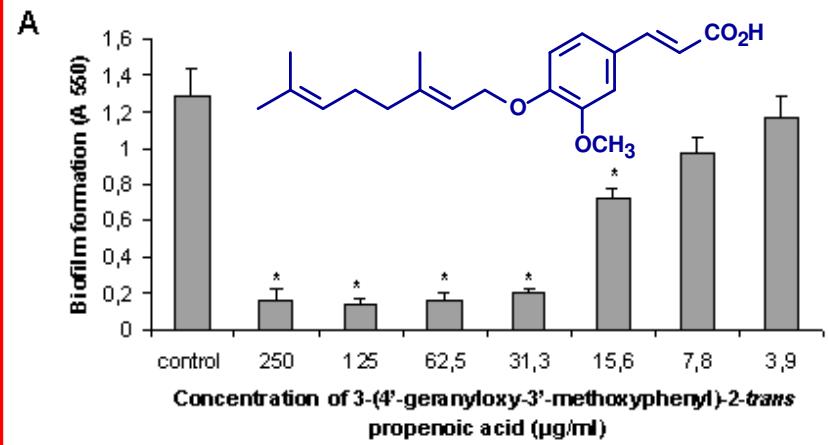


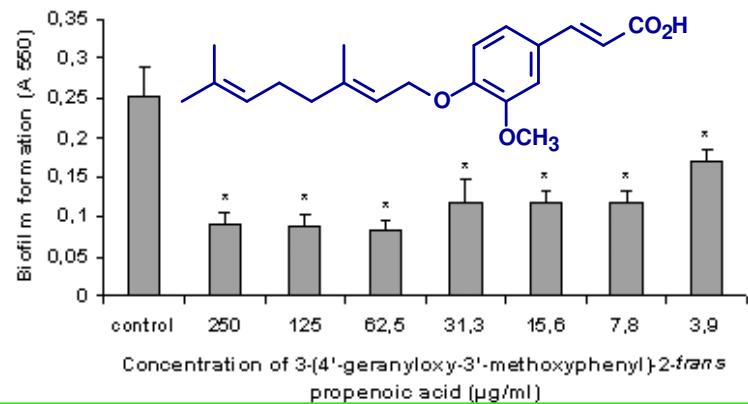
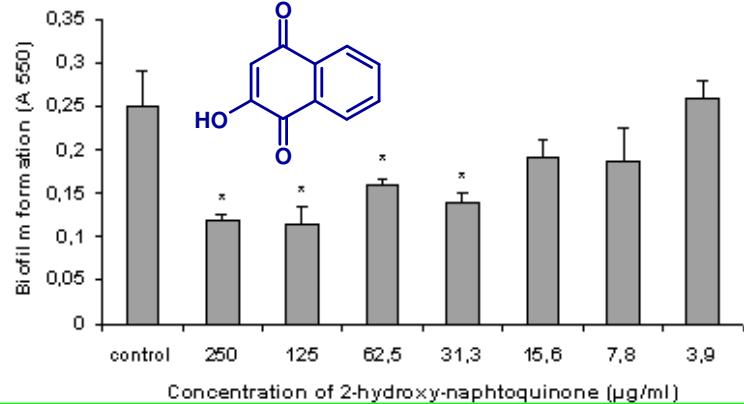
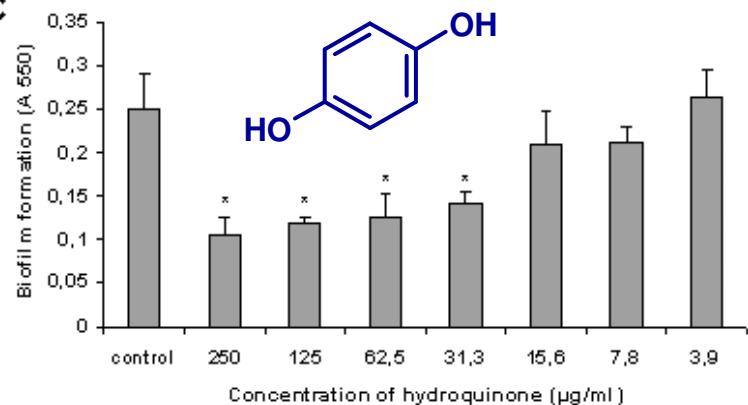
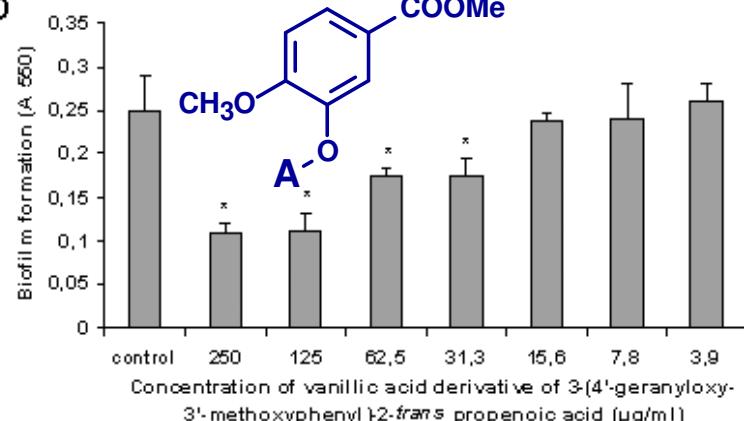
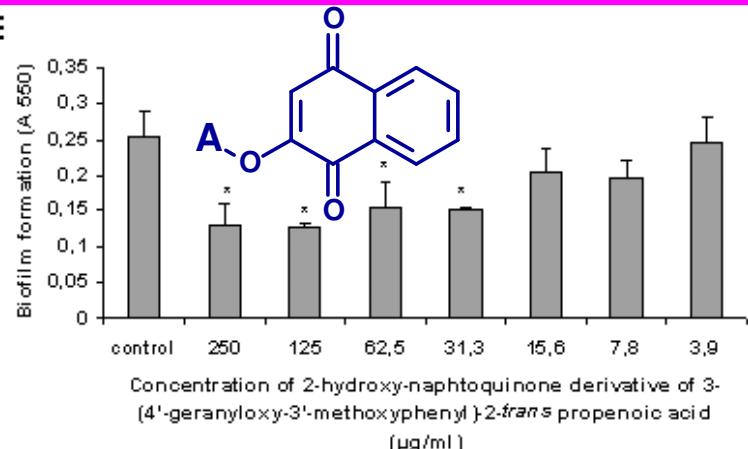
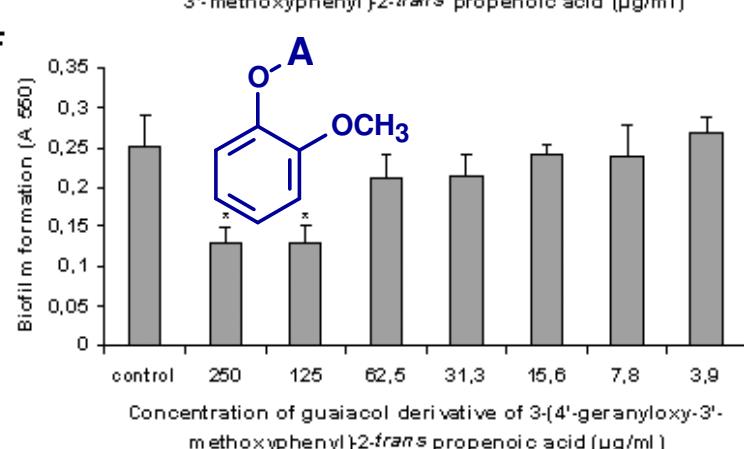
Gingivitis



Periodontitis





**A****B****C****D****E****F**

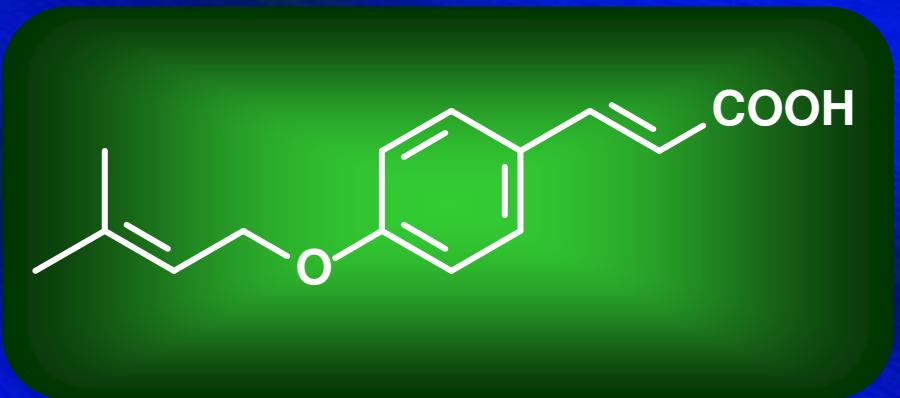
## Boropinic acid

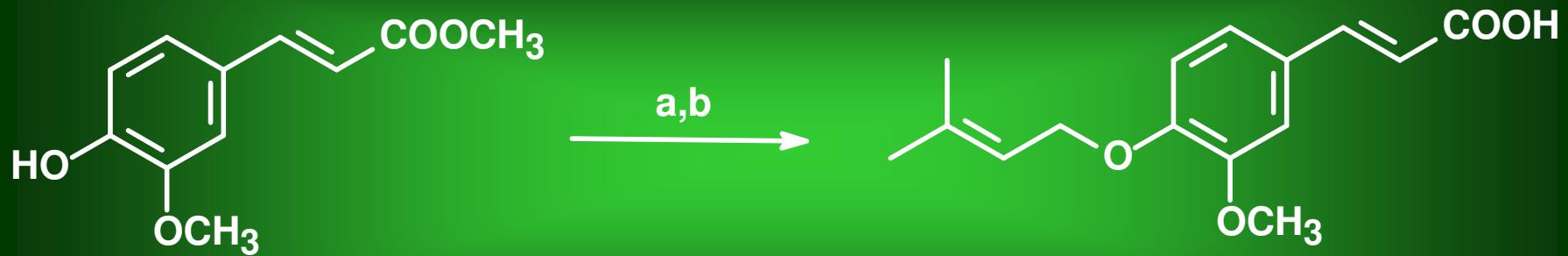


*Boronia pinnata* Sm.  
(Rutaceae)



*Esenbeckia hieronymi*  
(Rutaceae)





96 %

a:  $K_2CO_3$ , isopentenyl bromide, acetone  $60\ ^\circ C$ , 2h; b:  $NaOH$ ,  $60\ ^\circ C$ , 2h

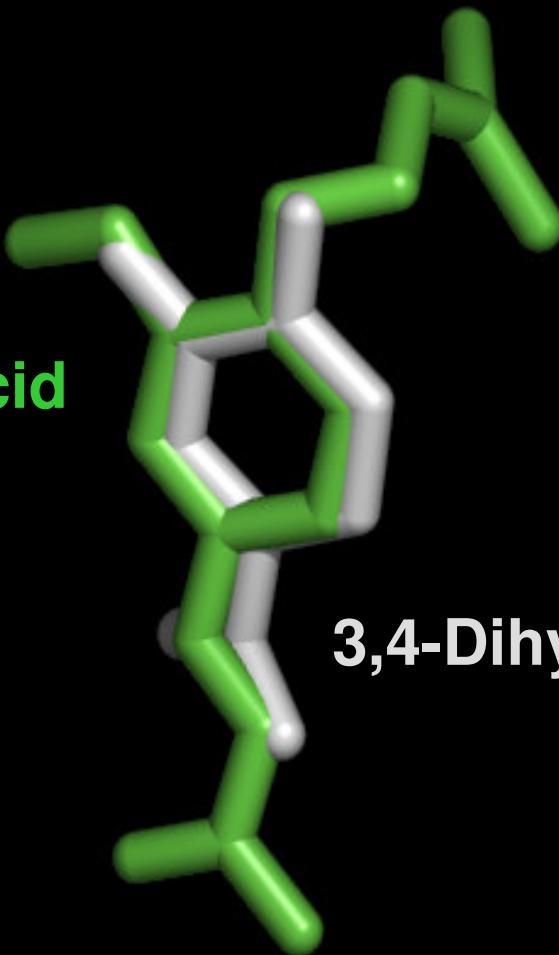
## Inhibition of soybean 5-lipoxygenase *in vitro*

	IC <sub>50</sub> ( $\mu\text{mol/mL}$ )*
4'-Geranyloxyferulic acid	0.262 $\pm$ 0.00220
Boropinic acid	2.89 $\times$ 10 <sup>-5</sup> $\pm$ 2.62 $\times$ 10 <sup>-6</sup>
Isopentenyloxycoumaric acid	0.007 $\pm$ 0,0004
Ascorbic acid	0.105 $\pm$ 0.0072
BHT	0.023 $\pm$ 0.0052
Trolox	0.047 $\pm$ 0.0048

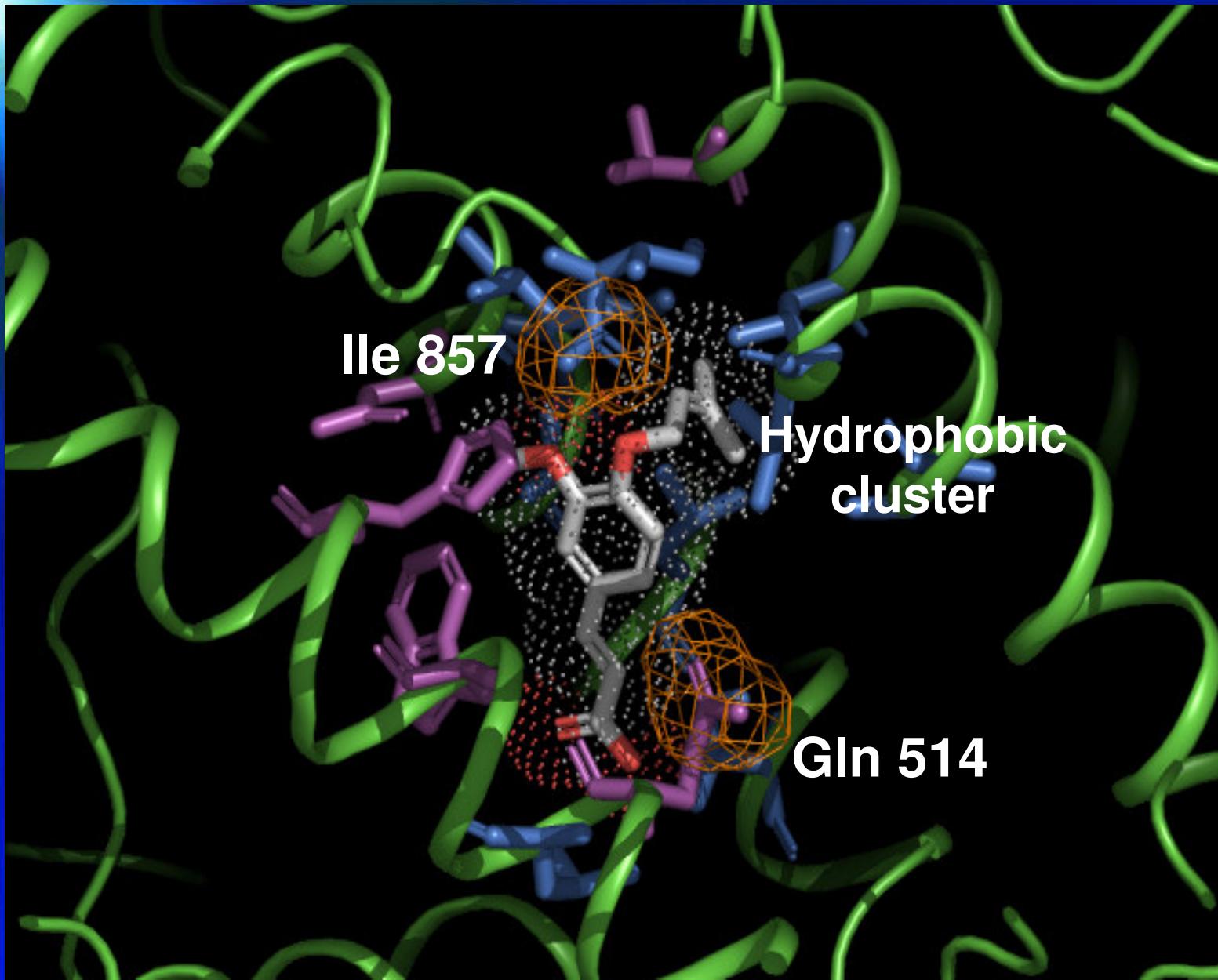
\* p < 0.05 Student's *t*-test

Curini, M.; Epifano, F.; Genovese, S.; Menghini, L.; Ricci, D.; Fraternale, D.; Giamperi, L.; Bucchini, A.; Bellacchio, E.  
*Nat. Prod. Commun.* 2006, 1, 1141

**Boropinic acid**



**3,4-Dihydroxybenzoic  
acid**



## Inhibition of the Croton oil-induced ear oedema in mice

Compound	N° an.	Oedema (mg) Mean ± S.E.	% Red.
Controls	10	6.9 ± 0.4	--
Boropinic acid	10	3.3± 0.2*	52
<i>p</i> -Isopentenyloxycoumaric acid	10	4.1 ± 0.4*	41
Nordihydroguaiaretic acid	10	4.4 ± 0.4*	36

\*  $p < 0.05$  Student's *t*-test

# Inhibition of growth of *Helicobacter pylori* (strain DSMZ 4867) *in vitro*

	MIC ( $\mu\text{g/mL}$ )*
Auraptene	50
4'-Geranyloxyferulic acid	> 200
Boropinic acid	1.62
Metronidazole	> 200
Tetracycline	4.00
Amoxycilline	0.78
Clarytromycine	1.25

\*Mean value from 3 experiments