

# Hepatoprotectants

≈hepatics

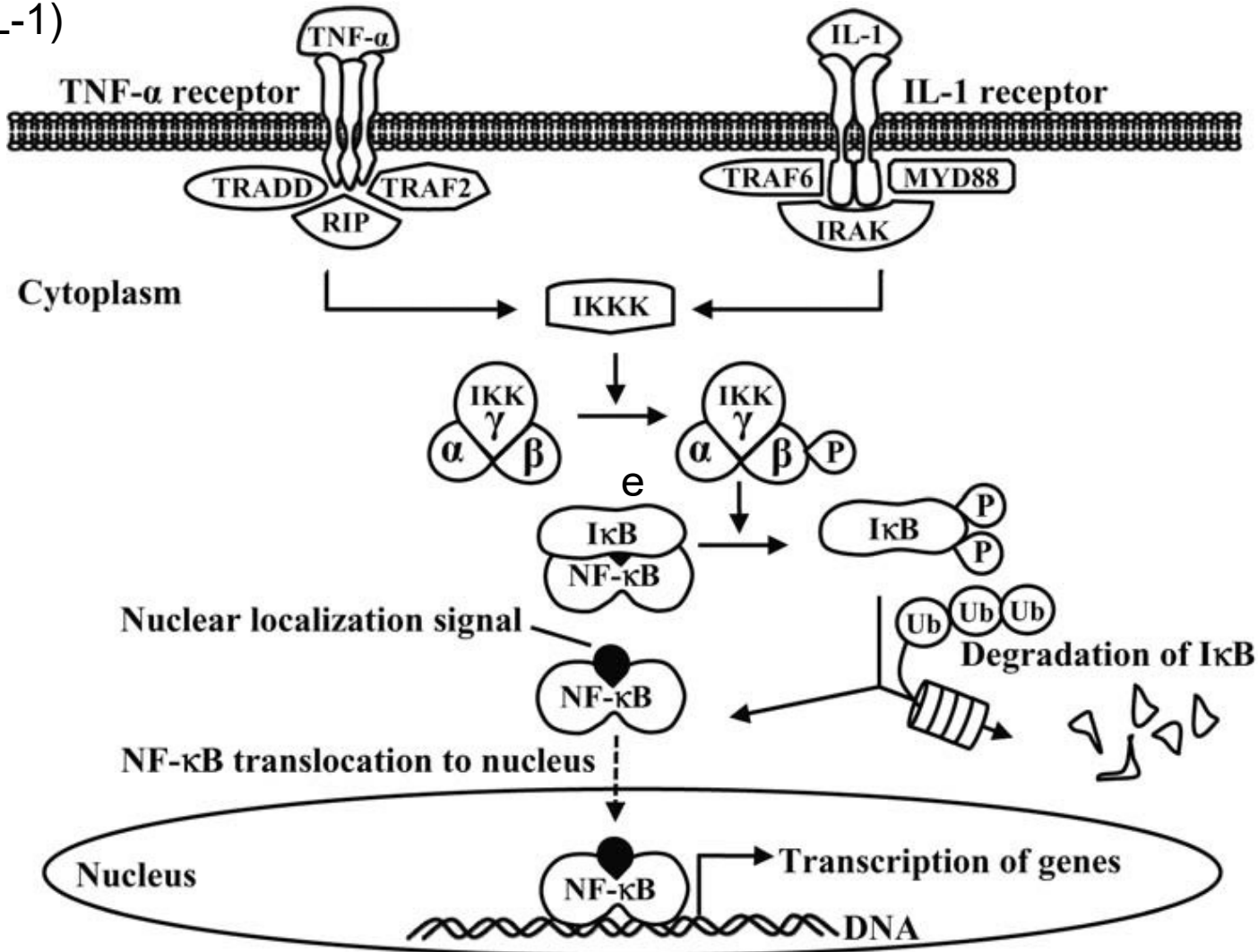
1. Inhibitors of nuclear transcription factor B (NF- $\kappa$ B)
  2. Antifibrotics
  3. Antioxidants
  4. Compounds which interfere with apoptosis
- most of compounds have multiple mechanisms of action

## Nuclear transcription factor B (NF- $\kappa$ B)

= a protein activating the immunity response of Kupfer cells of liver to harmful stimuli

- permanent or excessive activation leads to unwanted changes of liver tissue (cirrhosis, fibrosis)
- under different circumstances, activation of NF- $\kappa$ B can lead also to liver regeneration

# Activation of nuclear transcription factor B (NF- $\kappa$ B) by tumor necrosis factor $\alpha$ (TNF- $\alpha$ ) and interleukin 1 (IL-1)



NF- $\kappa$ B is in the cytoplasm in its inactive form linked with protein I $\kappa$ B (inhibitor  $\kappa$ B); this interaction disables transfer of NF- $\kappa$ B into the cell nucleus. NF- $\kappa$ B is activated if TNF- $\alpha$  or IL-1 are bound to their receptors, that leads to activation of intracellular signals and adaptor proteins, such as MyD88 (gene of primary myeloid differentiation response 88), IRAK (IL-1R-associated kinase) and TRAF-6 (TNF-associated factor 6) for receptor IL-1 and TRADD (TNF-associated protein of death domain), RIP (receptor-interacting protein) and TRAF2 (TNF-associated factor 2) for receptor TNF- $\alpha$ . These changes enable activation of IKKK (kinase of I $\kappa$ B kinase), which phosphorylates and activates IKK (I $\kappa$ B kinase), consisted from regulation subunit (IKK- $\gamma$ ) and two kinase subunits (IKK- $\alpha$ , IKK- $\beta$ ), that are responsible for phosphorylation of I $\kappa$ B. Then I $\kappa$ B is degraded by nuclear localisation signal and free NF- $\kappa$ B reaches the nucleus where it is bound to  $\kappa$ B enhancing elements of target gene and induce their transcription.

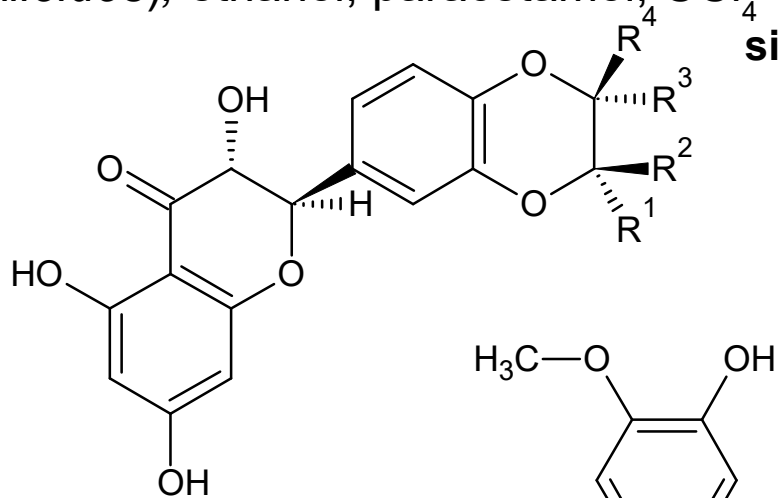
## Silymarin

= a mixture of flavanolignans gained by extraction of seeds of milk thistle (*Silybum marianum*; first referred by Pliny the Elder (= *Gaius Plinius Secundus* (23 AD – August 25, 79 AD) in 77 AD

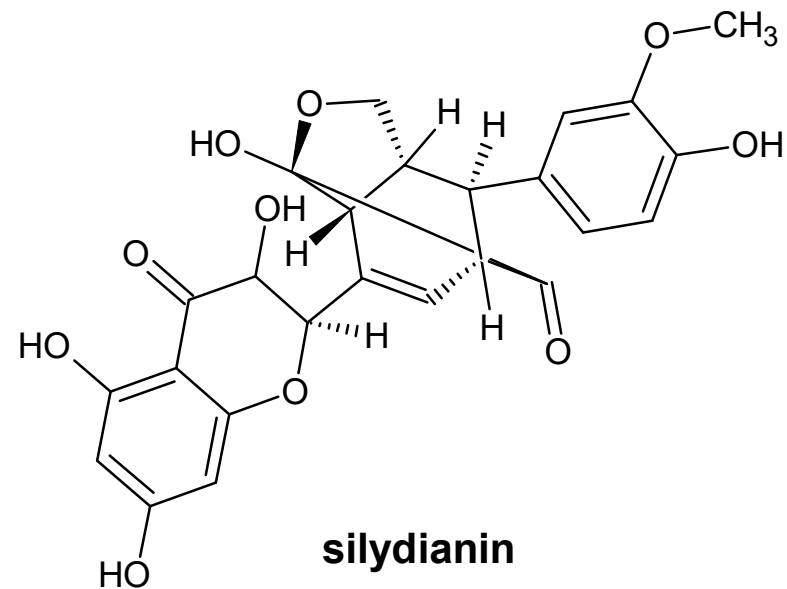
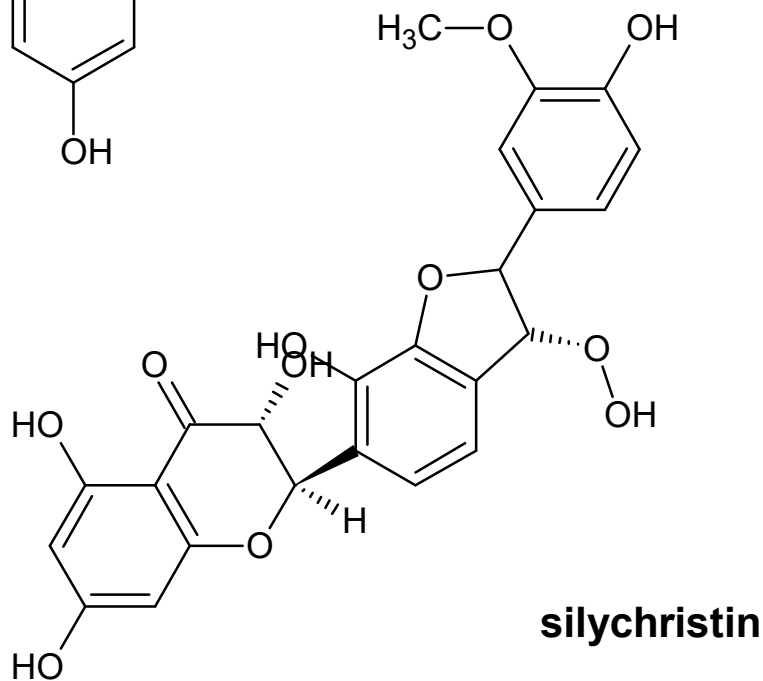
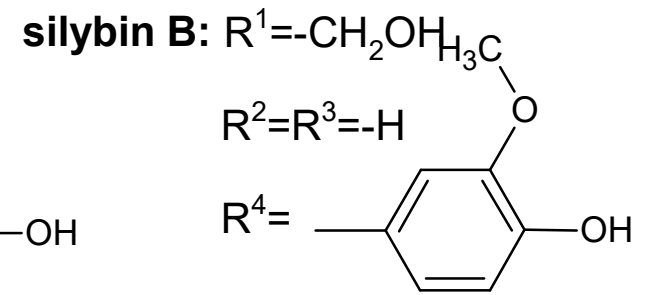
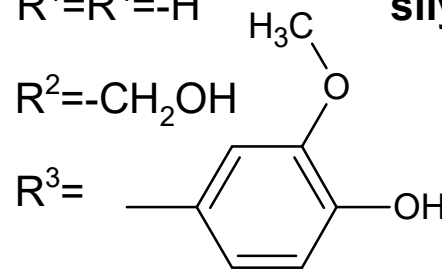
- content in seeds 1.5 – 3.5 %

- most of hepatoprotective activity is attributed to silybine (A+B) = silibinin; it represents 60 – 70 % of silymarin

- in silybin, hepatoprotective activity in liver damage by death cap (mushroom) (*Amanita phalloides*), ethanol, paracetamol,  $\text{CCl}_4$  etc. was demonstrated.

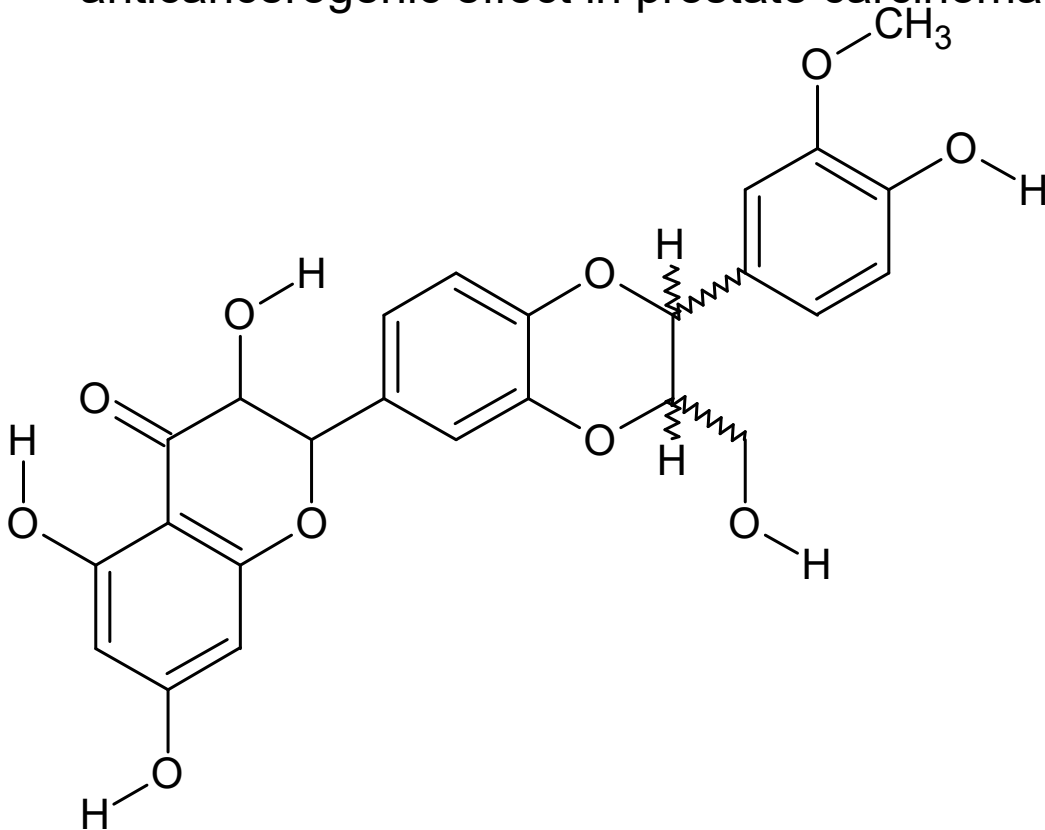


**silybin A:**  $\text{R}^1=\text{R}^4=-\text{H}$



## Effects of silymarin and their mechanisms

- inhibition of activation of NF- $\kappa$ B was demonstrated on hepatoma and lymphoma cells; probably main mechanism of action
- antioxidation effect: enhances superoxide dismutase activity in lymphocytes and erythrocytes, inhibits lipoperoxidation
- increases glutathione level
- anticancerogenic effect in prostate carcinoma



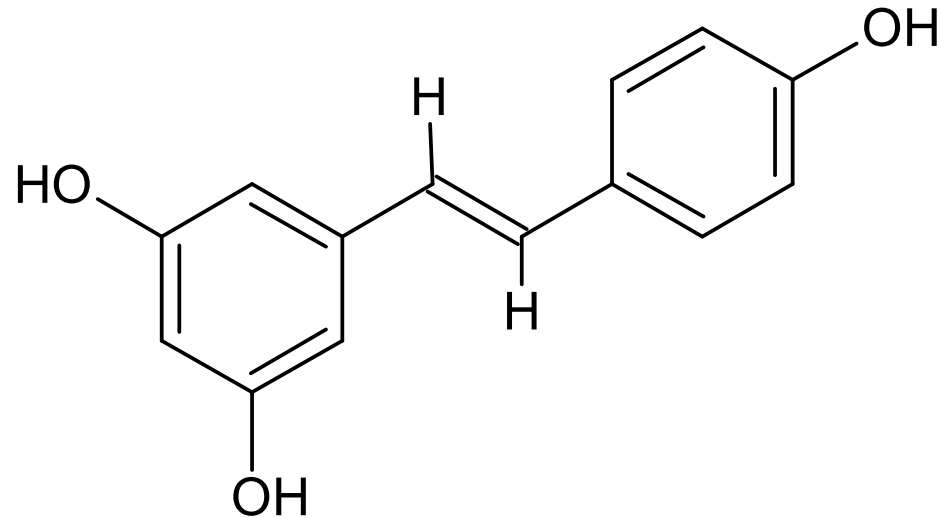
*PhEur: Silybi mariani extractum siccum  
rafinatum et normatum*

- silkristin + silidianin 20 – 45 %
- silibinin A + B 40 – 65 %
- isosilibinin A + B 10 – 20 %

### **isosilybin A+B (= isosilibinin A+B)**

- preparations Flavobion<sup>®</sup>, Lagosa<sup>®</sup>, Legalon<sup>®</sup>, Silygal<sup>®</sup>, Silymarin AL 50<sup>®</sup>

## Resveratrol

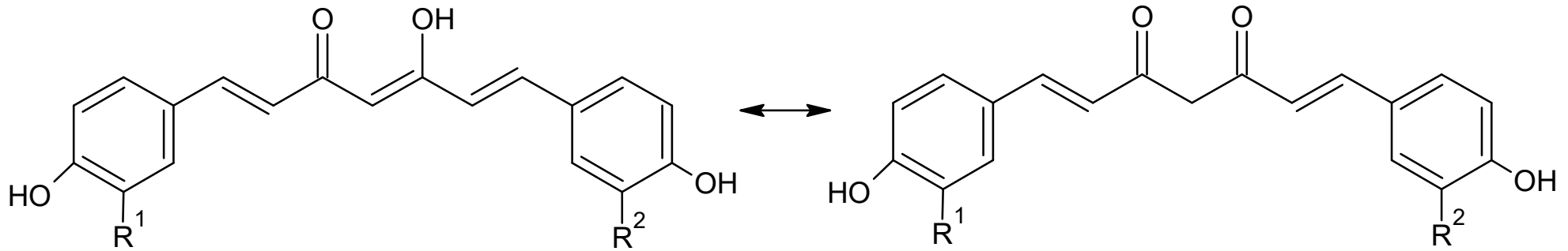


5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol  
3,5,4'-*trans*-trihydroxystilbene  
**resveratrol**

- *Arachis* (peanut), *Vitis vinifera* (grapevine)
- effects: antioxidant, anti-inflammatory, cancer prevention
- prevention of fibrose development
- protection before paracetamol toxicity and fibrosis caused by tetrachloromethane was demonstrated *in vitro*
- methylation of -OH does not decrease protective effects *in vivo*
- mechanism of action: inhibition of NF- $\kappa$ B activation

## Curcuminoids

• *Curcuma longa*, Zingiberaceae



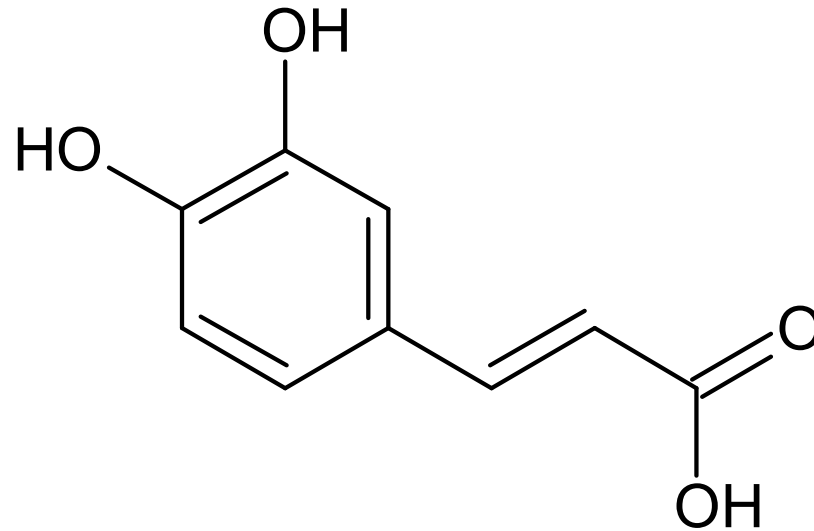
R<sup>1</sup>=R<sup>2</sup>=-OCH<sub>3</sub>      **curcumin**

R<sup>1</sup>=-H R<sup>2</sup>=-CH<sub>3</sub>      **demethoxycurcumin**

R<sup>1</sup>=R<sup>2</sup>=-H      **bisdemethoxycurcumin** (syn. curcumin III)

- mechanisms of action : inhibition of NF- $\kappa$ B, TNF- $\alpha$  and IL-1 $\beta$
- strong antioxidant activity, scavengers of many ROS
- lower cell membrane peroxidation
- curcumin is also the approved food additive (E 100, C.I. 75300)

Caffeic acid



3-(2,3-dihydroxyphenyl)prop-2-enoic acid

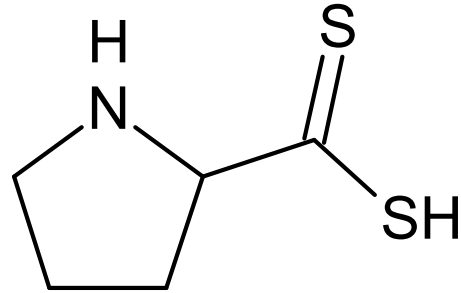
**caffeic acid**

•protection against damage by  $\text{CCl}_4$

- mechanisms of action:
1. inhibition of lipoxygenase 5 (which produces leucotriens damaging the liver)
  2. inhibition NF- $\kappa$ B activation
  3. free radicals scavenging

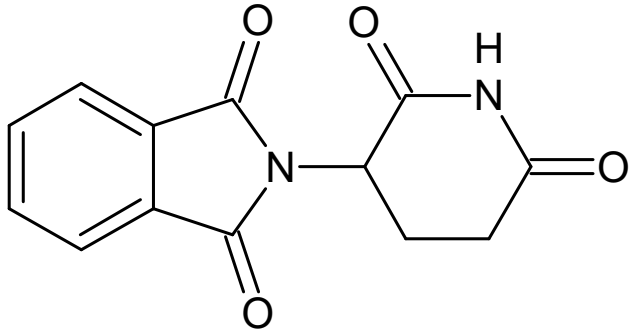


## Pyrrolidine-2-carbodithioic acid

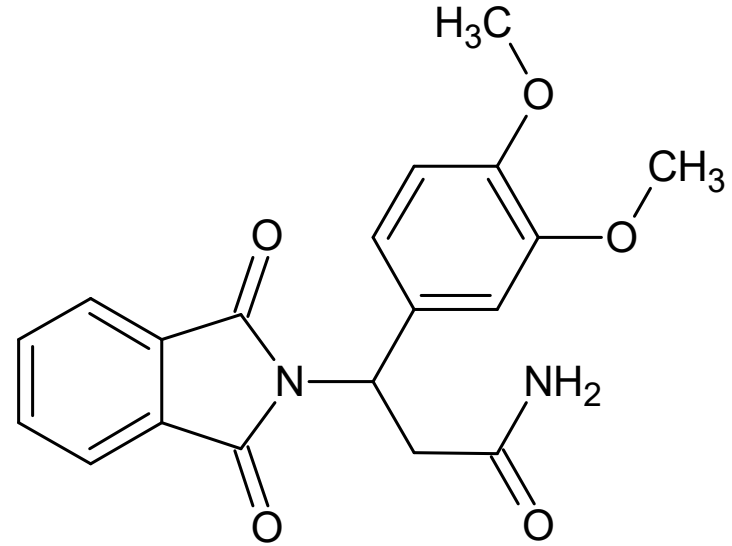


- syn. pyrrolidine-2-dithiocarboxylic acid, “**pyrrolidine dithiocarbamate**“, „**prolinedithiocarbamate**“, **PDTC**, dithioprolin
- known at least since 1958 (Zuman, Zahradník)
- mechanisms of action:
  1. antioxidant by complexation of metal cations which catalyse generation of free radicals
  2. inhibits activation of NF- $\kappa$ B

## Thalidomide and its analogues



2-(2,6-dioxopiperidine-3-yl)-1*H*-isoindole-1,3(2*H*)-dione  
 $\alpha$ -(*N*-phthalimido)glutarimide  
**thalidomide**

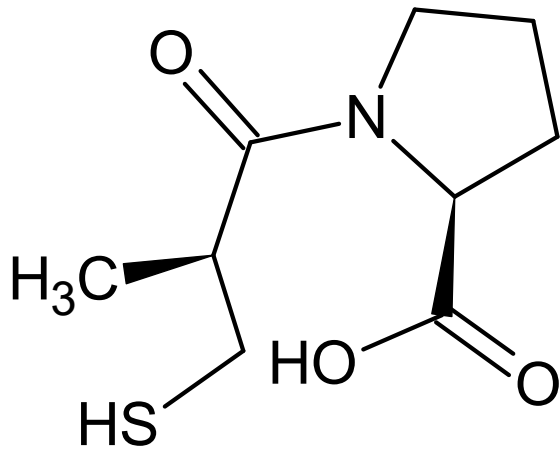


3-(3,4-dimethoxyphenyl)-3-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propanamide  
3-(phthalimido)-3-(3,4-dimethoxyphenyl)propanamide  
**PDP**

- originally hypnotic
- strong teratogene (Contergan<sup>®</sup>)
- abandoned in 1970<sup>th</sup> , now used in cancer therapy
- anti-inflammatory, antifibrotic and anticirrhotic activity
- an efficient inhibitor of NF- $\kappa$ B

## 2. Antifibrotics

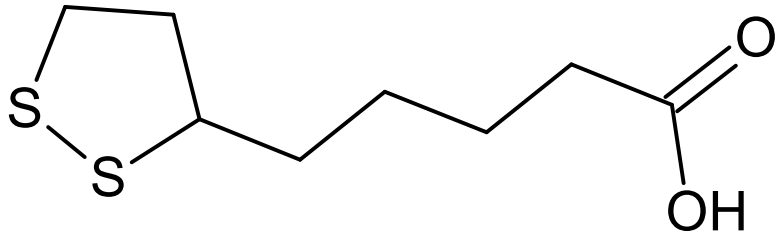
- angiotensin II (AT-II) a ACE play probably important roles in formation of liver fibrose
- transforming growth factor  $\beta$  (TGF- $\beta$ ) plays a dominat role in fibrose initiation; it can be supported by AT-II
- angiotensin receptor 1 antagonists lowers the portal pressure in hepatic cirrhosis
- hypothesis: inhibition of AT-II leads to NF- $\kappa$ B inactivation



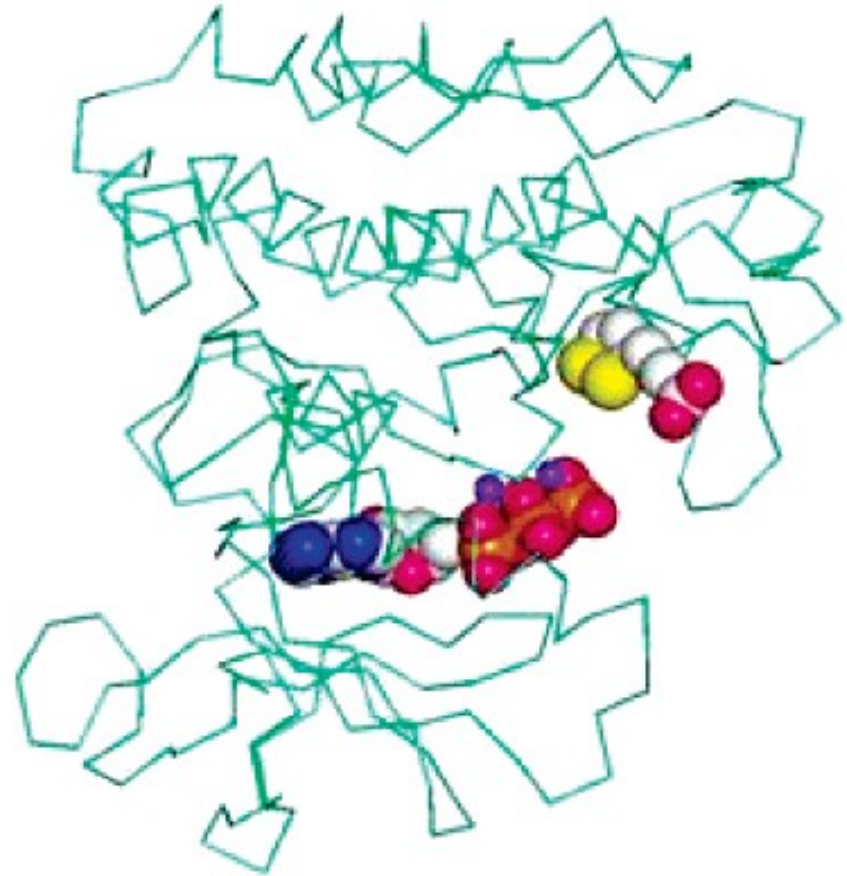
(2S)-1-[(2S)-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid  
**captopril**

(normally used as an anti-hypertensive agent)

### 3. Antioxidants

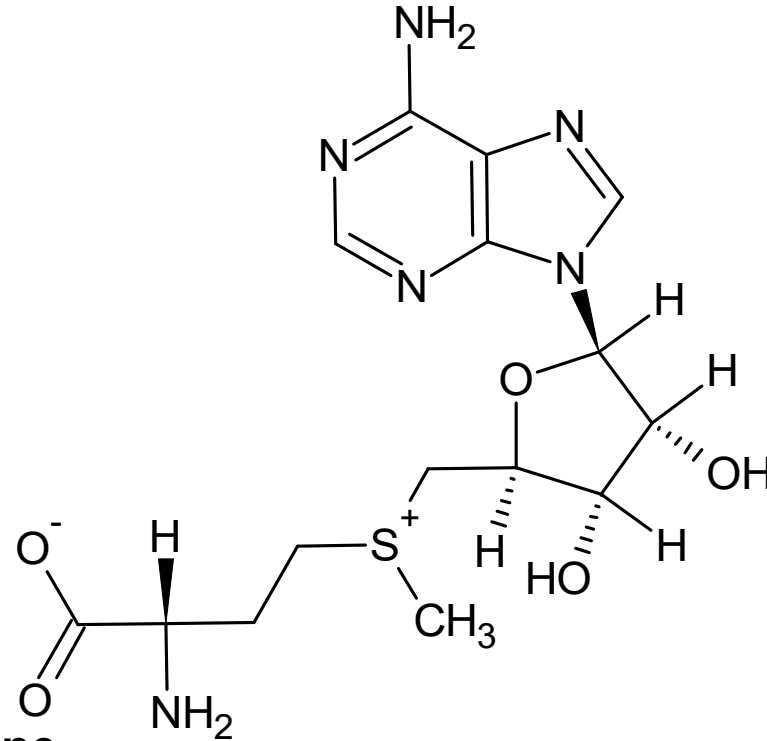


5-(1,2-dithiolan-3-yl)pentanoic acid  
**thioctic acid**  
lipoic acid



- inhibition of apoptosis of hepatocytes which had been induced by actinomycine D and TNF- $\alpha$  was demonstrated
- mechanism of action: activation of the insulin receptor by binding to tyrosinkinase domain
- used and authorised for long time as a drug for diabetic polyneuropathy (Thioktacid<sup>®</sup>, Thiogamma<sup>®</sup>)

## 4. Compounds which interfere with apoptosis

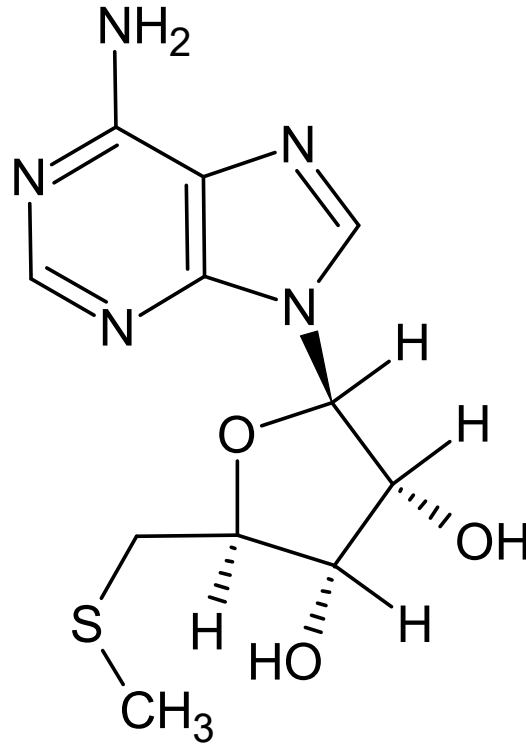


### **S-adenosylmethionine**

SAME, SAM, AdoMet

- endogenous compound, a donor of methyl
- synthesized from Met and ATP by the reaction catalyzed by methionine adenosyltransferase (MAT)
- regulates liver growth
- anti-apoptotic in normal cells, induces apoptosis in cancer cells; a mechanism of action related to proteins Bcl-x was proposed (Bcl-x belong to BCL-2 family, members of this family are central regulators of apoptosis); posttranslational splicing of Bcl-x protein can lead to Bcl-x<sub>L</sub>, that is anti-apoptotic, or to Bcl-x<sub>S</sub> which is proapoptotic; SAME and methionyladenosin (MTA) induced selectively Bcl-x<sub>S</sub> in HepG2 cancer cells; the alternative splicing is modulated by protein phosphatase 1 (PP1) and its inhibitors block the ability of SAME and MTA to induce Bcl-x<sub>S</sub>

- SAME and MTA increased the amount of mRNA for the catalytic subunit PP1 in HepG2 cells, but not in normal hepatocytes
- SAME is freely available in food supplements in the USA



### **methylthioadenosine (MTA)**

5'-deoxy-5'-methylsulfanyladenine

- a side product of SAME metabolism gained in polyamines synthesis

# The hepatal metabolism of S-adenosylmethionine (SAME)

