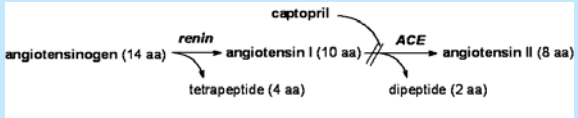
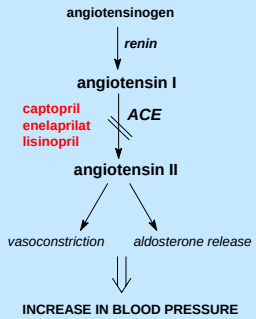
Angiotensin converting enzyme (ACE)

ACE is a zinc-containing *dipeptidyl carboxypeptidase*, which converts **angiotensin I** to **angiotensin II**

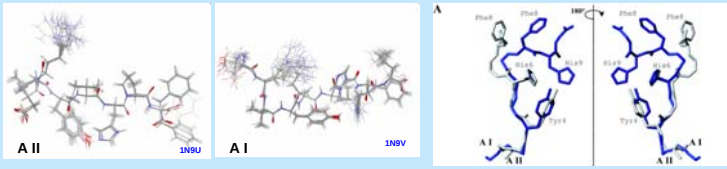


Angiotensin II is an octapeptide that **contracts blood vessels** and increases salt and water reabsorption in the kidney (mainly by stimulating the release of **aldosterone**), resulting in the **increase of blood pressure**

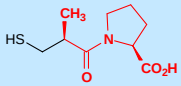
Inhibitors of ACE, like **captopril, enalaprilat** and **lisinopril** are important **antihypertensive drugs**



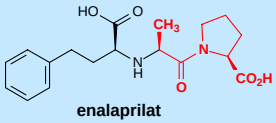
Angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu)



Solution structures of angiotensin I & II by NMR



captopril

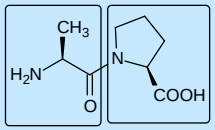


enalaprilat

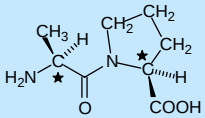
lisinopril

Captopril, enalaprilat and **lisinopril** are **antihypertensive drugs** used to lower high blood pressure in hypertensive individuals. Their mechanism of action is inhibition of **angiotensin converting enzyme (ACE)**

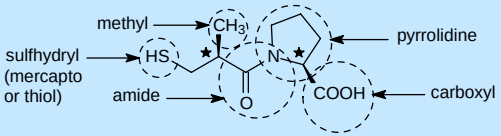
Chemistry of captopril



L-Ala — L-Pro



(*S*)-Ala — (*S*)-Pro



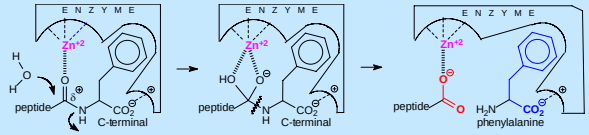
captopril

Captopril was developed (by **Ondetti** and **Cushman** at Squibb), using rational design, based on (1) the analogy of its target **ACE** to **carboxypeptidase A**, a much studied enzyme, and (2) the structure of its inhibitor, **benzylsuccinate**

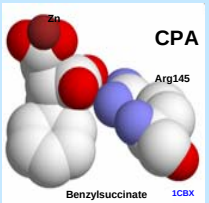
Carboxypeptidase A (CPA)

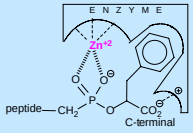
A proteolytic enzyme with a catalytic **zinc** at the active site

Selectively **cleaves at a C-terminal aromatic residue**, e.g., phenylalanine

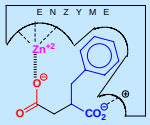


substrate — terahedral intermediate — products still bound to the active site

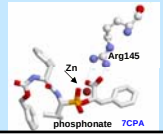


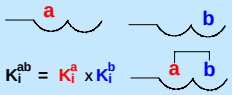


phosphonate (transition-state analog)

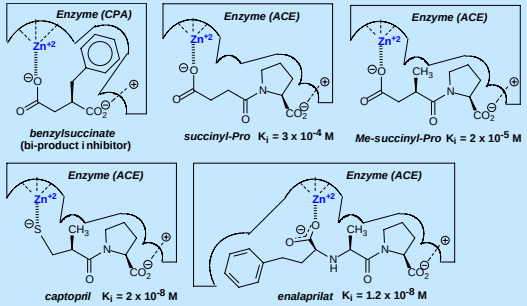


benzylsuccinate (bi-product inhibitor)



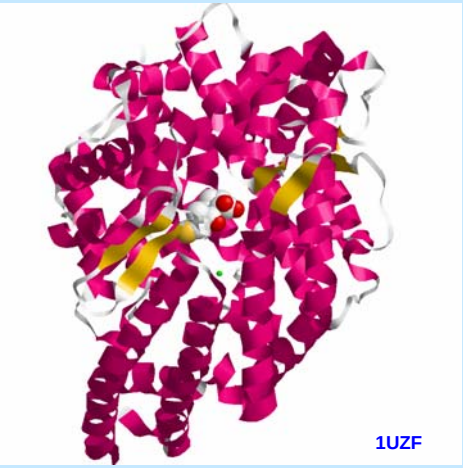


$K_i^{ab} = K_i^a \times K_i^b$

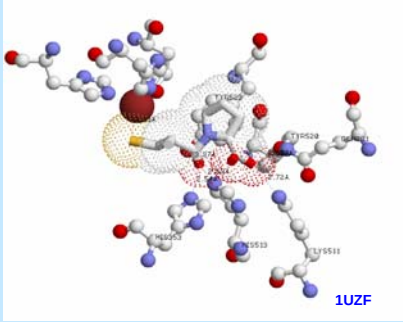


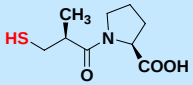
Based on the potent CPA inhibitory activity of benzylsuccinic acid, **succinyl-proline** was designed as an **inhibitor of ACE** - it had modest activity (K_i 0.3 mM) The CH_3-group (Ala side-chain) was attached that improved the K_i (0.02 mM). The COOH-group was replaced by an SH-group for stronger binding to the active site **Zn** leading to **captopril** with a superior K_i-value of 2 x 10^{-8} M (0.00002 mM) and potent *in vivo* activity. **Enalaprilat** (K_i-value of 1.2 x 10^{-8} M) was subsequently developed to **replace the oxidizable SH-group with a COOH**. In addition, an **NH-** and a **phenylethyl-group** were inserted to **compensate for the loss of the strong Zn-S bond**

Complex of captopril with angiotensin converting enzyme



Captopril bound to the active site of ACE

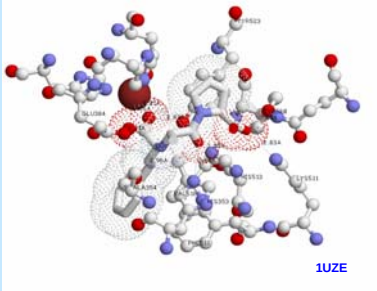


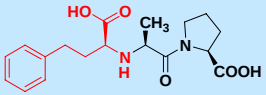


$K_i = 2 \times 10^{-8}$ M

- The **thiol (SH) group** of **captopril** makes direct interaction with **Zn^{2+}**
- The **carbonyl group** forms strong **hydrogen bonding** with **two histidines, His 513 and His353**
- One oxygen of **proline carboxylate group** is **H-bonded** to **Tyr 520, Gln 281 and Lys 511**

Enalaprilat bound to the active site of ACE

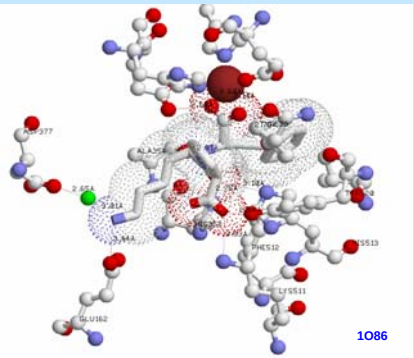


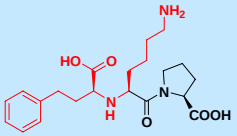


$K_i = 1.2 \times 10^{-8}$ M

- **Side effects of captopril lead to the design of enalaprilat**
- The **carboxylate** group introduced to **replace the SH-group** binds to **Zn^{2+}** as well as to **Glu 384 and Tyr 523**
- The **carbonyl** group attached to proline forms strong **H-bonds** with **two histidines, His 513 and His 353,** similarly to captopril
- One oxygen of **proline carboxylate** group is **H-bonded** to **Tyr 520, Gln 281 and Lys 511**, similarly to captopril
- The **carbonyl oxygen of Ala 354** forms a **H-bond** to the new **NH-group**
- **The phenylethyl side-chain** introduced occupies a **hydrophobic pocket lined by Phe 512 and Val 518**

Lisinopril bound to the active site of ACE





$K_i = <1 \times 10^{-9}$ M

In **lisinopril** the **alanine of enalaprilat** is replaced by **lysine.** The positively charged side-chain of lysine binds to **Glu 162** as well as to **Asp 377** *via* **a bridging water molecule** *(green)*