

2.,4. 11. 2010

Metabolizem zdravih učinkovin

Metabolizem ksenobiotikov

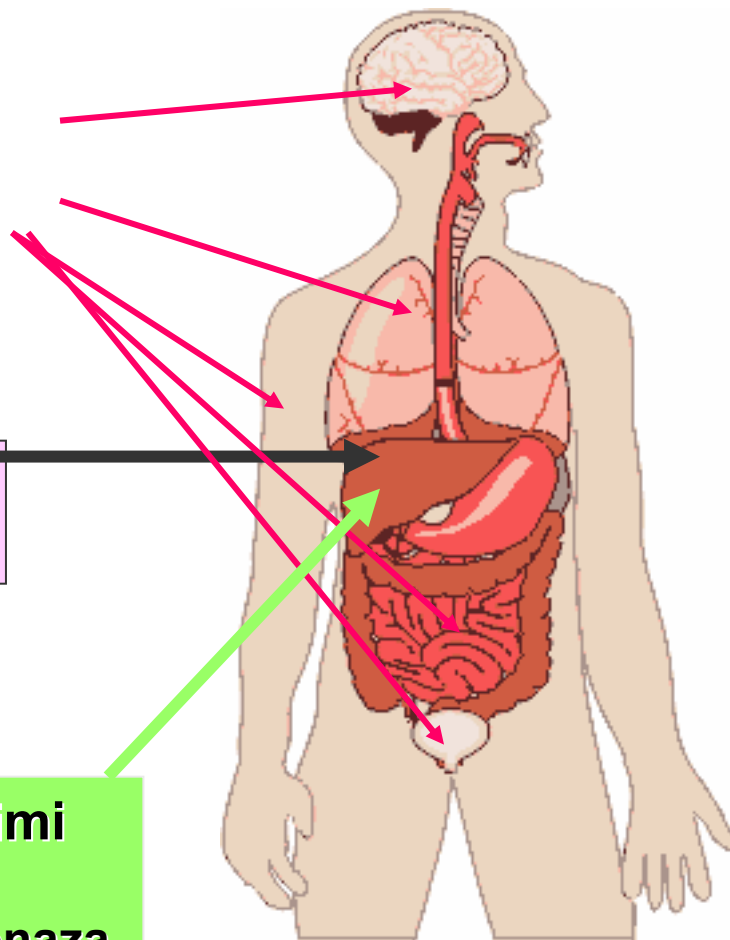
š. I. 2010/2011

Metabolizam učinkovin

**Ne-jetrni mikrosomani encimi
(oksidacija, konjugacija)**

**Jetrni mikrosomalni encimi
(oksidacija, konjugacija)**

**Jetrni ne-mikrosomalni encimi
(acetilacija, sulfatiranje, GSH,
alkoholna/aldehidna dehidrogenaza,
hidroliza, oksidacija/redukcija)**



Jetrni mikrosomalni sistem

- Oksidacije so katalizirane s Citohromom P450

CYT

- Primeri:

- Nastanek **neaktivnih polarnih** metabolitov
- Nastanek **aktivnih polarnih** metabolitov
- Nastanek **toksičnih** metabolitov
- Nastanek metabolitov z **drugačnimi farmakokinetickimi/farmakodinamskimi lastnostmi**

CYP Nomenklatura

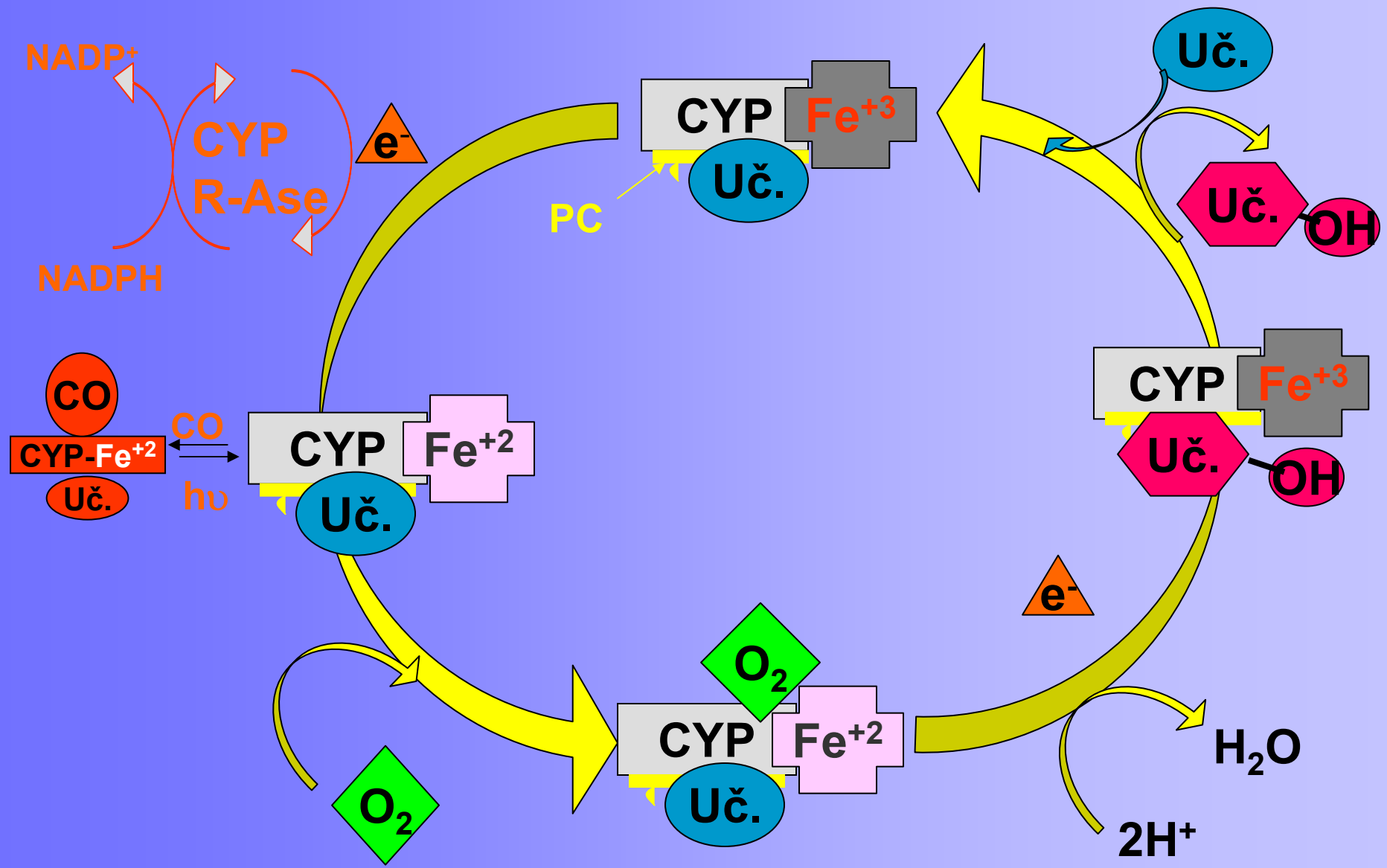
- **Družina:** CYP in arabska številka, npr.: **CYP1** (znotraj družine je >40% homologije zaporedja amino kislin).
- **Poddružina:** označimo z veliko črko za številko: npr.: **CYP1A**. (znotraj poddružine je 40-55% homologija)
- **Poddružina:** dodatna arabska številka, če je več kot 1 poddružina, npr.: **CYP1A2**
- *Če je ime zapisano v poševni pisavi je to ime gena: (**CYP1A2**).*

Družina citohromskih encimov **CYP**

- Obstaja **12 družin** CYP genov pri človeku; delitev na osnovi homolognosti encimov.
- Večina zdravilnih učinkovin se metabolizira z **CYP 1, 2, in 3** družinami.
- CYP encimi imajo molekulano maso okoli **45-60 kDa**.
- Pogosto 2 ali več encimov katalizira isti tip oksidacije: **široka substratna specifičnost**.
- **CYP3A4** je najobičajnejši encim pri metabolizmu učinkovin; njegova prisotnost v GIT je vzrok za majhno per os razpoložljivost mnogih učinkovin.

Izoencimi CYP P450 (CYPs): lastnosti

- $\text{NADPH} + \text{H}^+ + \text{O}_2 + \text{U} \rightarrow \text{NADP}^+ + \text{H}_2\text{O} + \text{U-OH}$
- CO se veže na reduciran Fe(II) hem (abs. pri 450 nm: ime encima)
- CYP je monooksigenazna družina encimov, ki je glavni katalizator oksidacije ksenobiotikov in endogenih spojin v jetrih, ledvicah, GIT, koži, pljučih.
- Oksidacija: CYP hemski protein, reduktaza, NADPH, fosfatidilholin and molekularni kisik.
- CYPs encimi so v gladkem endoplazmatskem retikulumu blizu skupaj z NADPH-CYP reduktazo v 10/1 razmerju
- Reduktaza je vir elektronov za pogon oksidacijskega kroga.



Tok electron pri mikrosomalni oksidaciji učinkovin

Človeški jetrni CYP encimi vpleteni v metabolizem učinkovin

CYP encim	delež (% od celote)	Območje variacije
1A2	~ 13	~ 40 krat
1B1	< 1	
2A6	~ 4	~ 30 – 100 krat
2B6	< 1	~ 50 krat
2C	~ 18	25-100 krat
2D6	Do 2.5	> 1000 krat
2E1	do 7	~ 20 krat
2F1		
2J2		
3A4	do 28	~ 20 -krat
4A, 4B		

Faktorji, ki vplivajo na aktivnost in nivo CYP encimov

Prehrana	1A1; 1A2 ; 2E1 ; 3A3; 3A4,5
Kajenje	1A1; 1A2
Alkohol	2E1
Učinkovine	1A1, 1A2; 2A6; 2B6; 2C ; 2D6 ; 3A3, 3A4,5
Okolje	1A1, 1A2 ; 2A6; 1B; 2E1 ; 3A3 , 3A4,5
Genetski polimorfizem	1A; 2A6; 2C9,19 ; 2D6 ; 2E1

Rdeče: encimi pomembni v metabolizmu učinkovin

Sodelovanje CYP encimov v metabolizmu nekaterih klinično pomembnih zdravilnih učinkovin -1

CYP Encim	sodelovanje v metabolizmu (%)	Primeri učinkovin
1B1		17 β -Estradiol
2F1	~1.3	Ipomeanol
4A		Prostaglandini
1A1	2.5	R-varfarin
2A6	2.5	Ciklofosfamid, Halotane Zidovudin, AZT
2B6	3.4	Ciklofosfamide, Testosteron
2E1	4.1	Acetaminofen, Klorzoksazon Dapson. Halotan
1A2	8.2	Acetaminofen, Kofein Fenacetin, (R) –varfarin

Sodelovanje CYP encimov v metabolizmu nekaterih klinično pomembnih zdravilnih učinkovin -2

CYP Encim	sodelovanje v metabolizmu (%)	Primeri učinkovin
2C8,9	15.8	Tolbutamide Dillofenak (S) –varfarin, Fenitoin Heksobarbital
2C18, 19	8.3	Diazepam, Omeprazol (S) –Mefenitoin
2D6	18.8	Kodein, Debrisokvin Dekstrometorfan “Ekstasi”, Bufuralol, Spartein
3A4,5	34.1	karbamazepine, Kortizol Dapsone, Diazepam Erithomicin, Midazolam Nifedipin, Omeprazol Testosteron

Učinkovine, ki zavirajo metabolizem učinkovin s tvorbo kompleksov s CYP

Amfetamin

Cimetidin

Dapson

2,5-Dimetoksi-4-metilamfetamin

Difenilhidramin

Eritromicin

Fenfluramine

Itrakonazol

Ketokonazol

Metadon

Metamfetamin

Nortriptilin

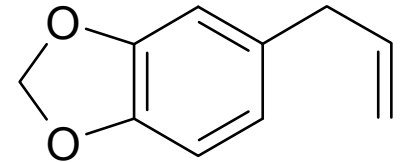
SKF 525A

Sulfanilamide

Snovi brez dušika, ki vplivajo na metabolizem učinkovin s tvorbo kompleksov s CYPs

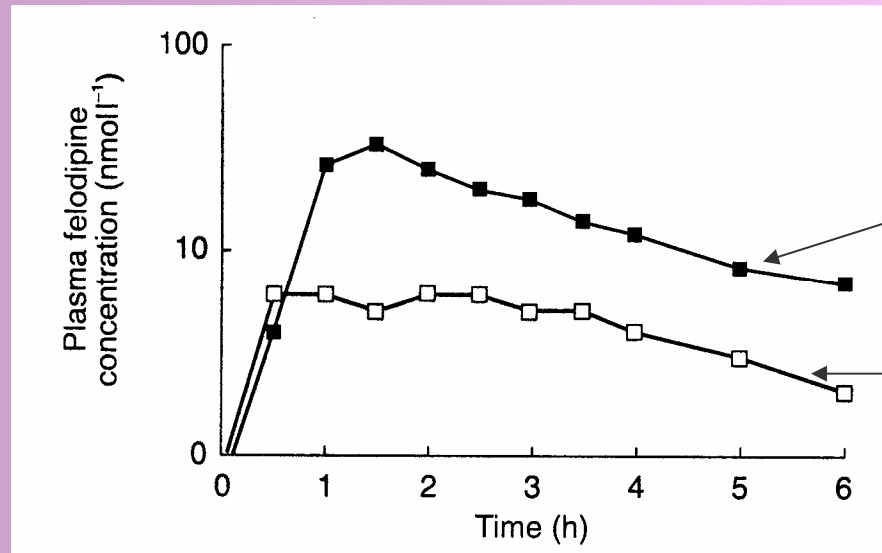
- **Sok grenivke** - CYP 3A4 inhibitor; zelo variabilen učinek; neznana snov

– D.G. Bailey, et al.; Br J Clin Pharmacol 1998, 46:101-110



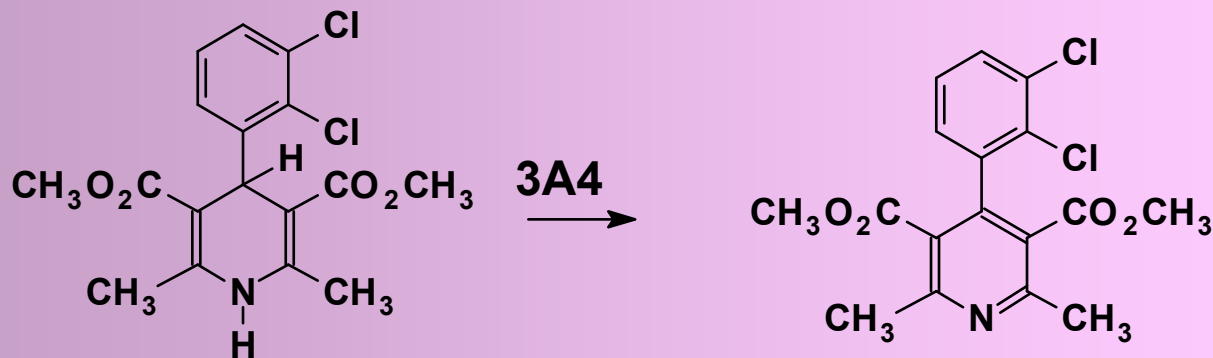
- **Izosafrole, safrole** - CYP1A1, CYP1A2 inhibitor; v nekaterih pivih
- **Piperonil butoksid in alkohol** -CYP1A1, CYP1A2 induktor; kot sestavina insekticidov

Vpliv soka grenivke na plazemsko koncentracijo Felodipina



5mg tableta
s sokom

Brez soka



Človeški CYP nejtrni encim udeleženi v metabolizmu - 1

CYP Encim	Tkivo
1A1	Pljuča, ledvica, GIT, koža, placenta
1B1	Koža, ledvica, prostata, dojke
2A6	Pljuča, nosna membrana
2B6	GIT, pljuča
2C	GIT (tanko črevo) grlo, pljuča
2D6	GIT

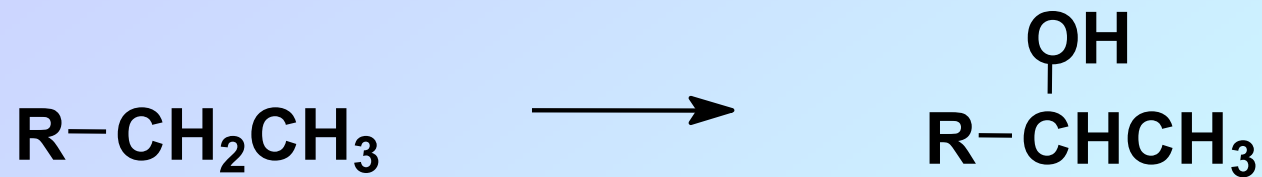
Človeški CYP nejetrni encim udeleženi v metabolizmu - 2

CYP encim	Tkivo
2E1	Pljuča, placenta
2F1	Pljuča, placenta
2J2	Srce
3A	GIT, pljuča, placenta, fetus, uterus, ledvica
4B1	Pljuča, placenta
4A11	Ledvica

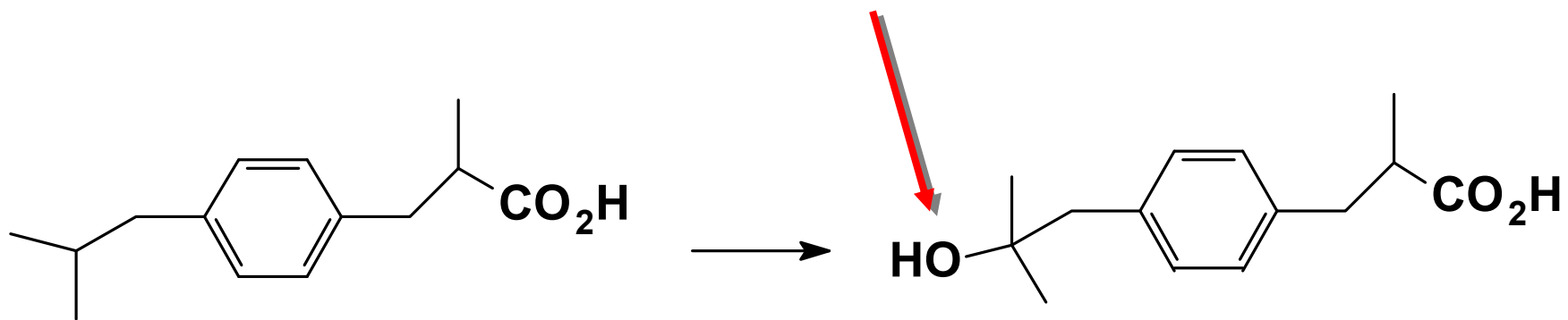
CYP Biotransformacije = metabolizem

- Kemično različne majhne molekule se pretvorijo v bolj polarne produkte
- Tipi reakcij:
 - **Alifatska hidroksiliranja,**
 - **Aromatska hidroksiliranja**
 - **Dealkiliranja (N-, O-, S-)**
 - **N-oksidacije, S-oksidacije**
 - **Deaminacije**
 - **Dehalogeniranja**

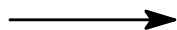
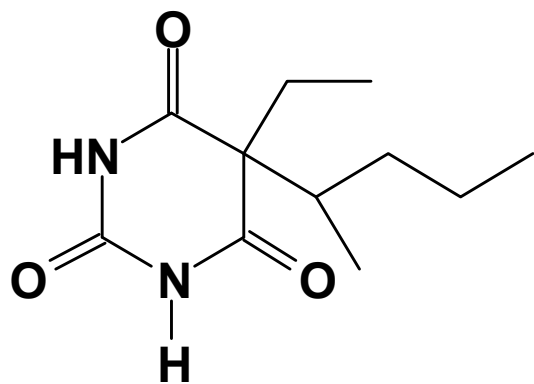
Alifatsko hidroksiliranje



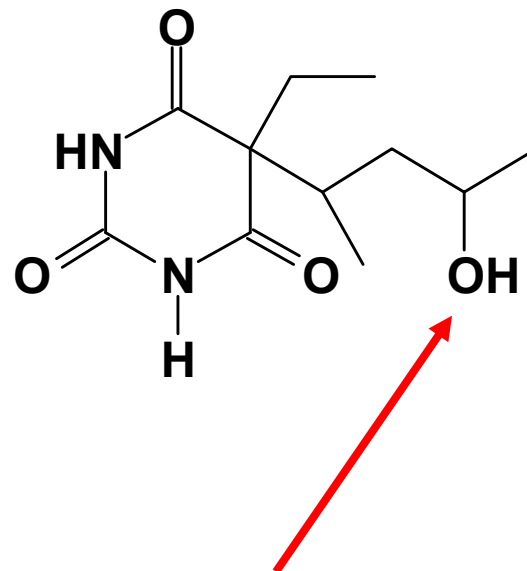
Primeri: ibuprofen, pentobarbital



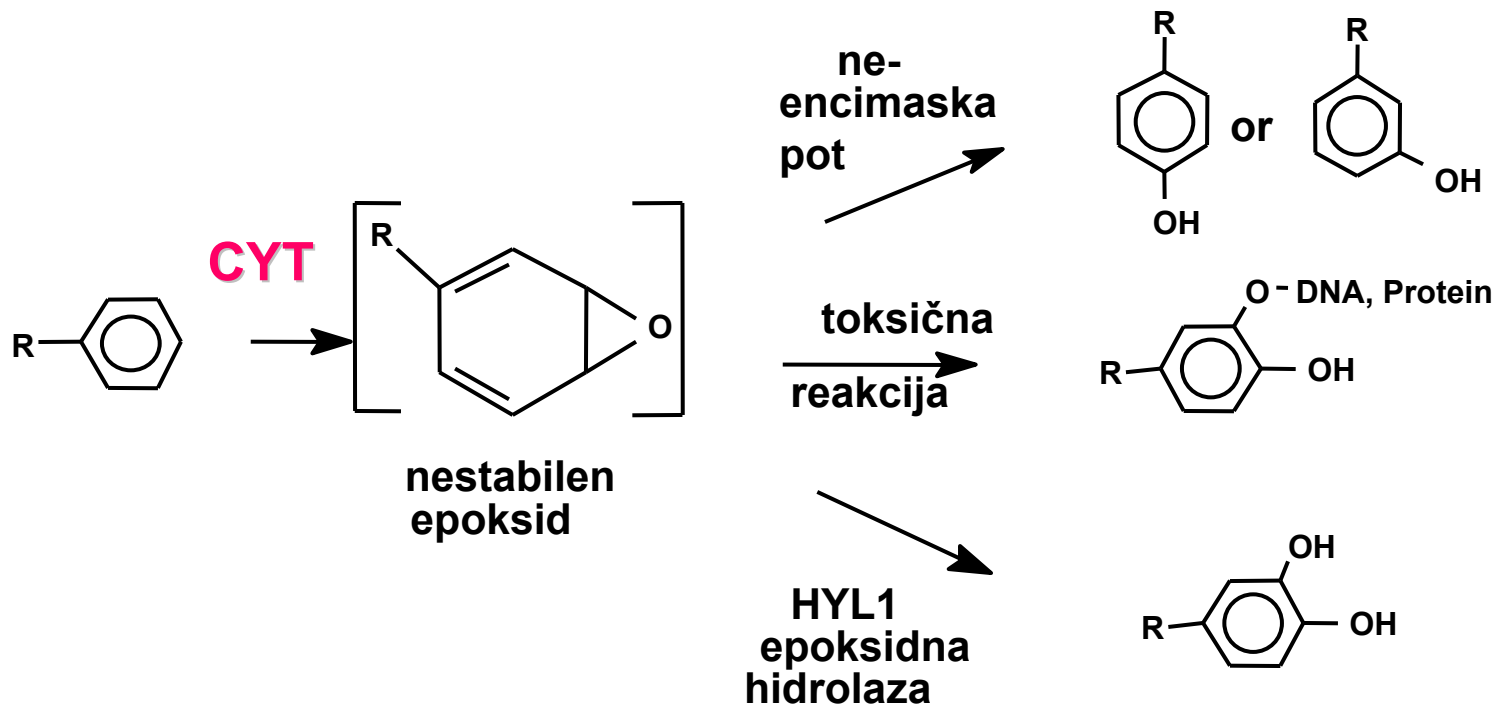
ibuprofen



pentobarbital

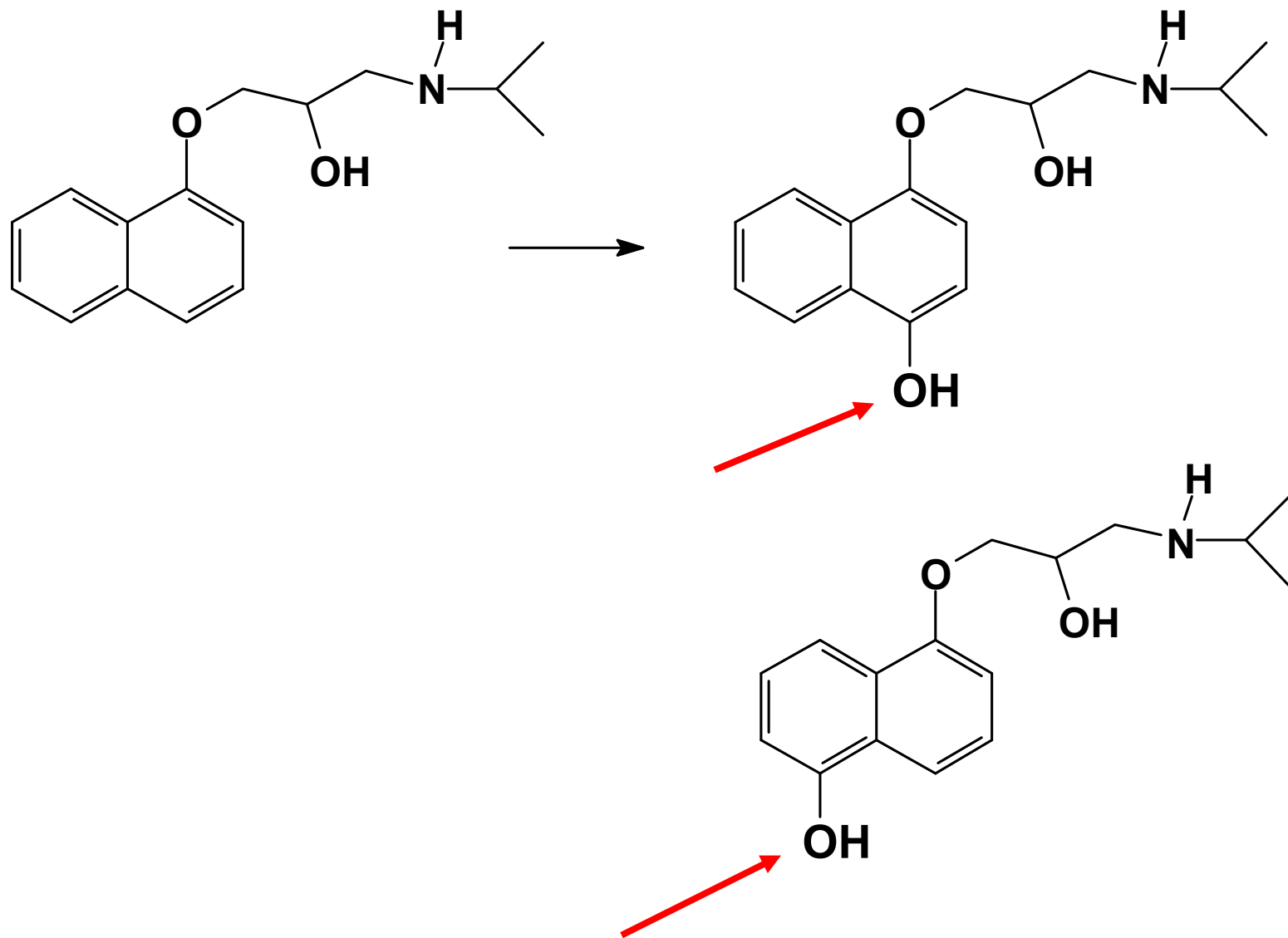


Aromatska hidroksilacija

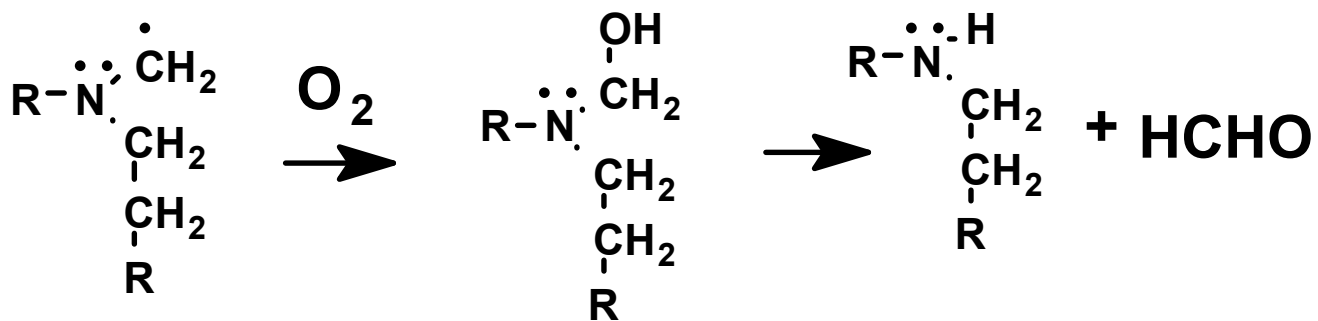
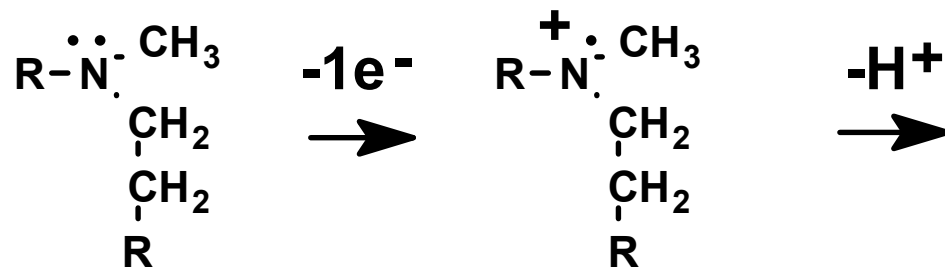


Primeri: acetanilid, fenitoin, propranolol
Endogene snovi: steroidni hormoni

propranolol

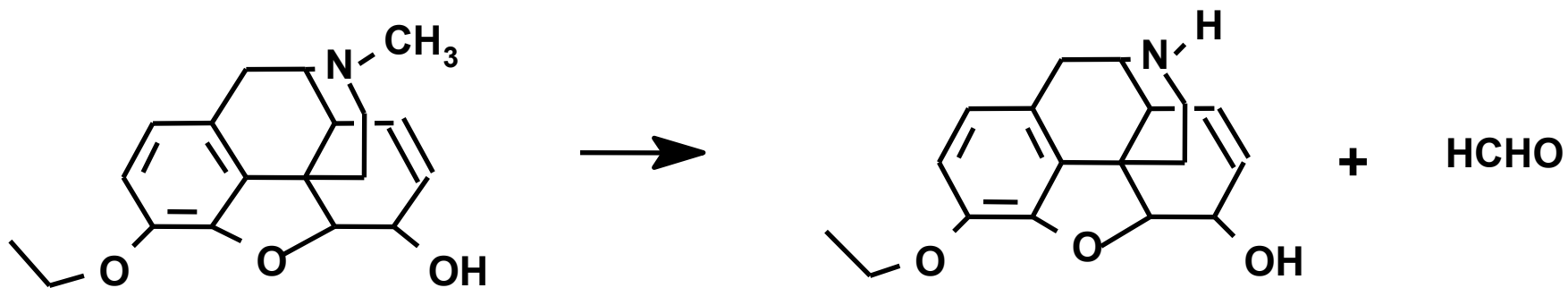


N (or O, S)-Dealkilacije



N-demetilacija da formaldehid

etilmorfin

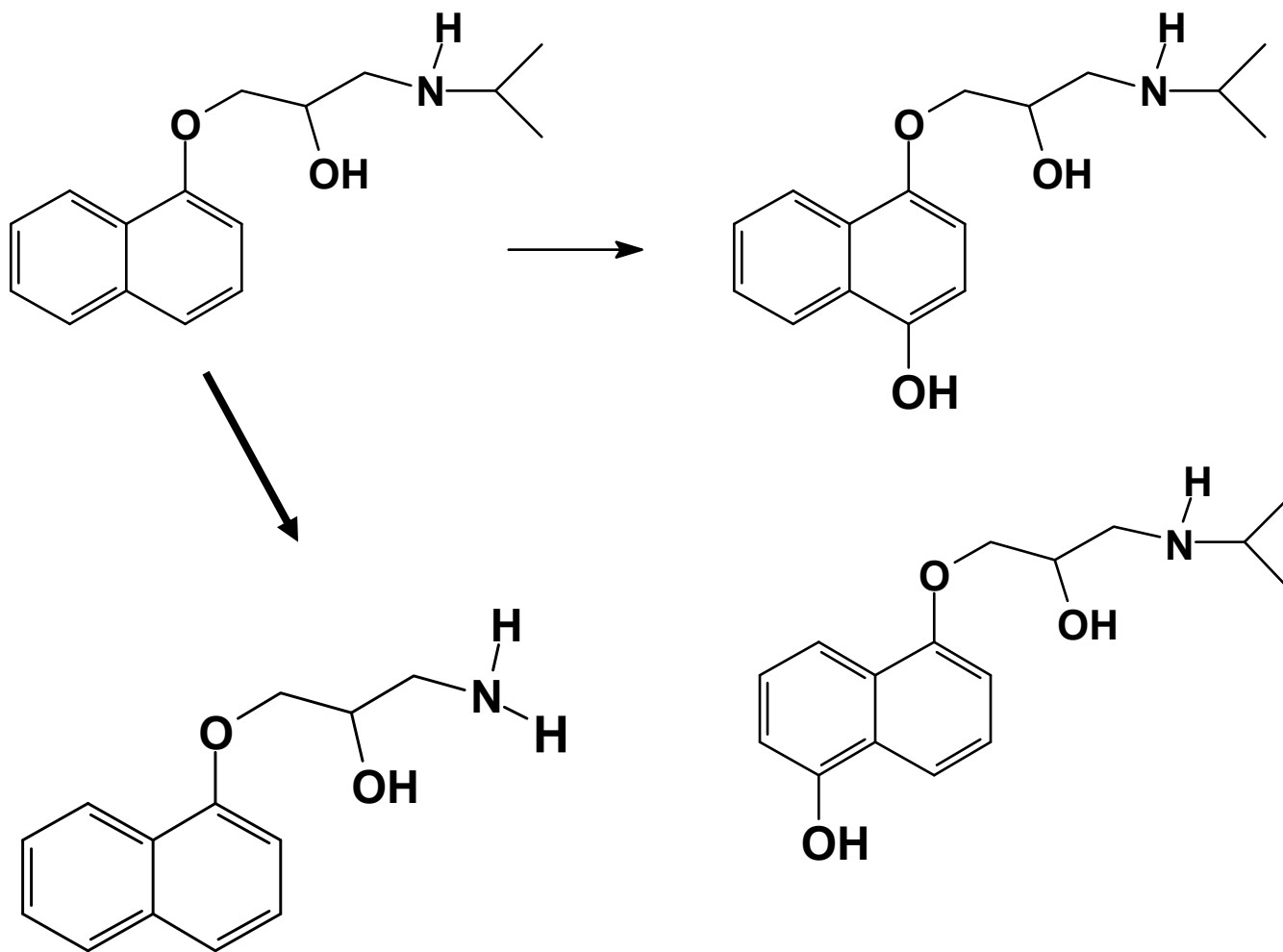


etilmorfin

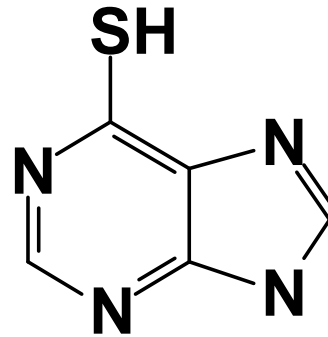
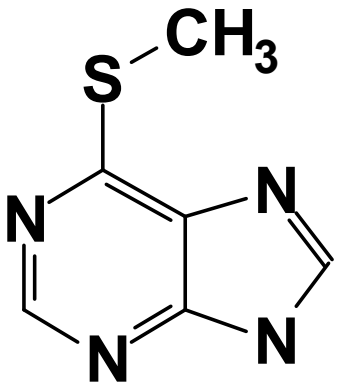
desmetil-etilmorfin

N-demetiliranje je prednostno do O-dealkiliranja

propranolol

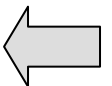


6-metiltiopurin

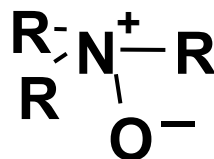
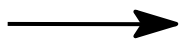
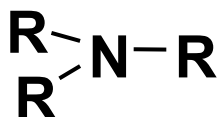
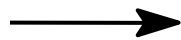


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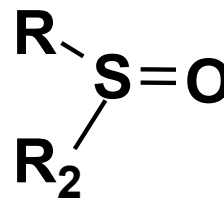


N-Oksidacije

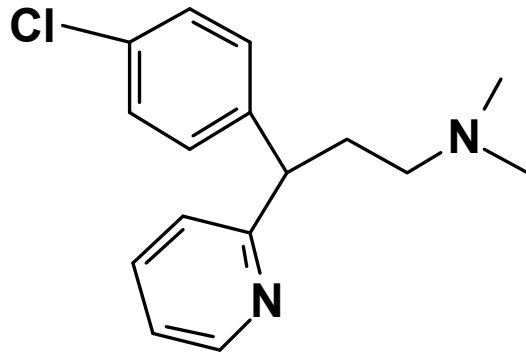


Primeri: klorfeniramin, trimetilamin

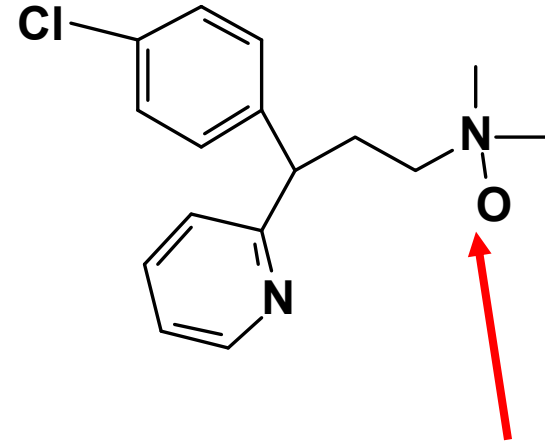
S-Oksidacije



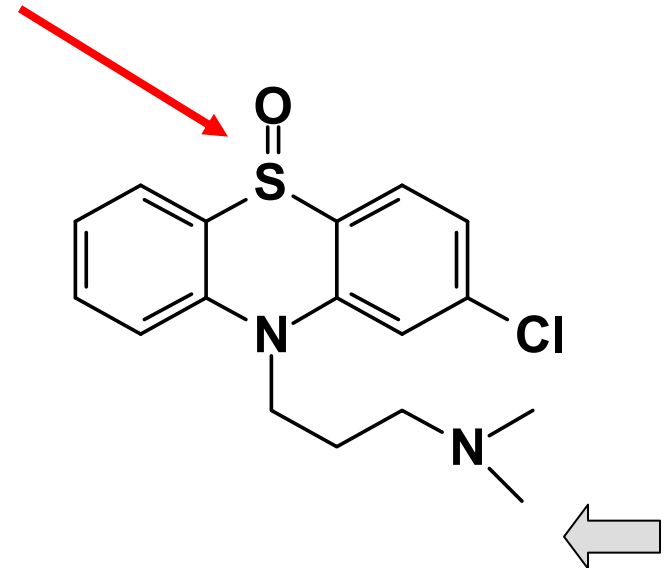
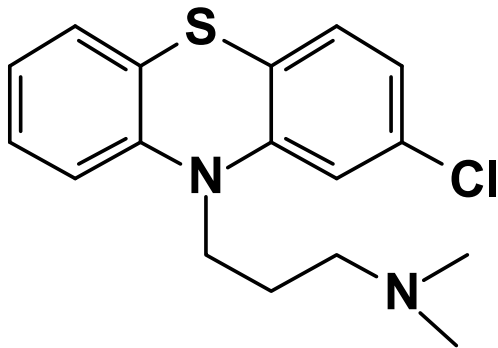
Primeri: klorpromazin, cimetidin



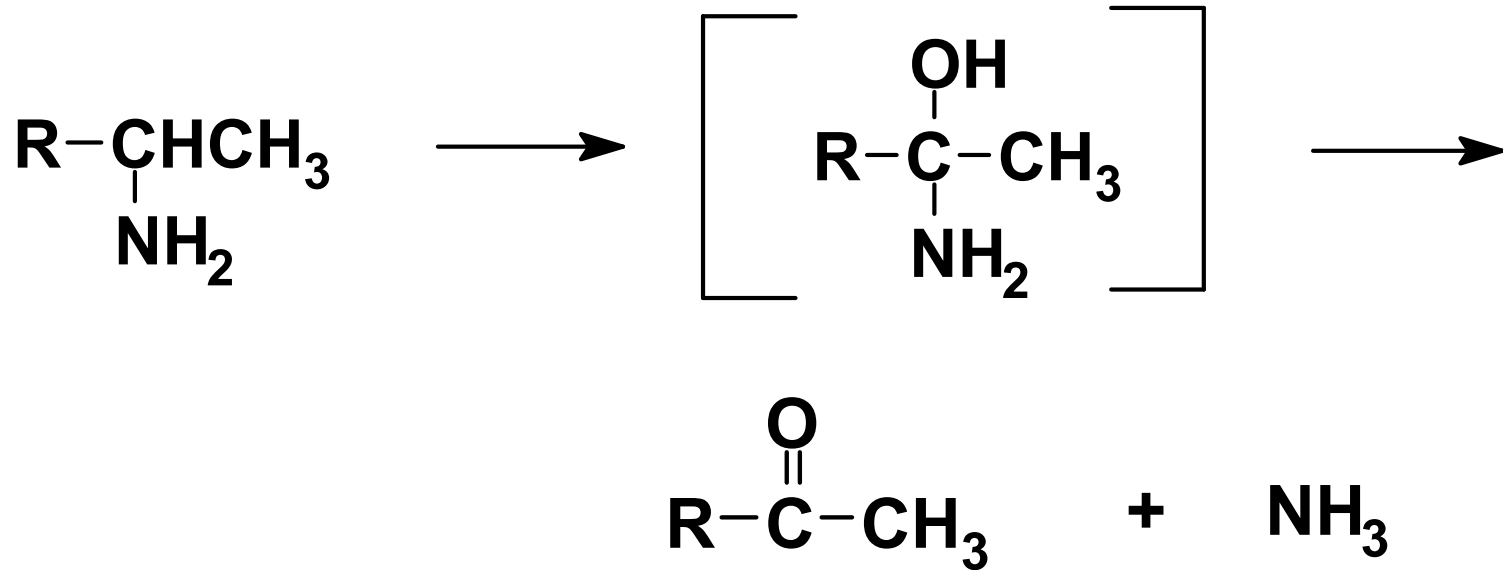
klorfeniramine



klorpromazin

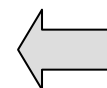
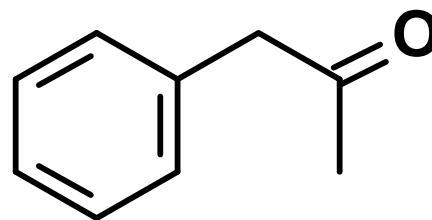
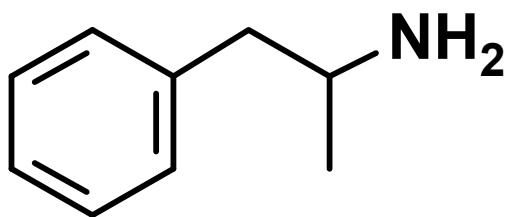


Deaminiranje

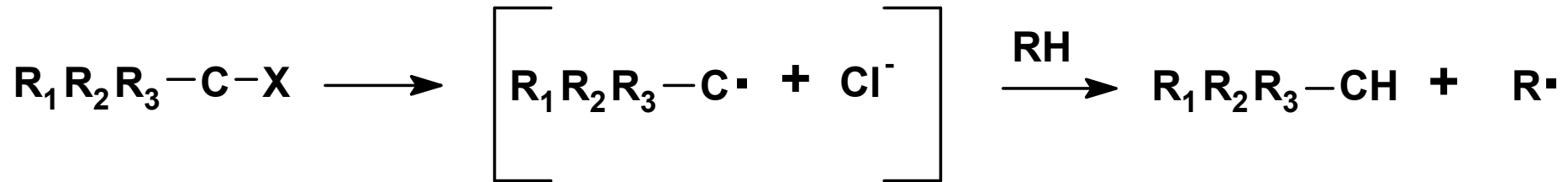


Primeri: amfetamin, diazepam

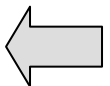
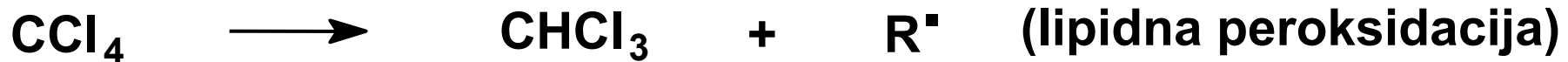
amphetamine



Dehalogeniranje

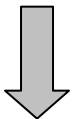


Primeri: ugljikov tetraklorid, halotane, metoksifluran



Biotransformacije, kjer CYP niso vpleteni

- Oksidacije
- Hidrolize
- **Konjugacije (Faza 2)**
 - Glavne reakcije konjugacije
 - Glukuronidacija (velika kapaciteta)
 - Sulfonacija (nizka kapaciteta)
 - Acetiliranje (spremenljiva kapaciteta)
 - Primeri: Prokainamid, Isoniazid
 - Druge reakcije konjugacije: O-metiliranje, S-metiliranje, aminokislinska konjugacija (glicin, taurine, glutation)



Ne-CYP oksidacija učinkovin - 1

- **Monoamino oksidaze (MAO), Diamino oksidaze (DAO)** - MAO (mitohondrijska) oksidativno deaminira endogene snovi (dopamin, serotonin, noradrenalin, adrenalin);

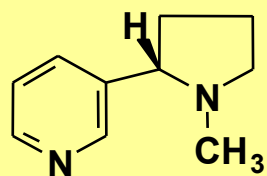
Učinkovine, ki inhibirajo MAO povečajo koncentracijo biogenih aminov v CŽS.

- **Alkoholna in aldehidna dehidrogenaza** - nespecifični encimi v topni frakciji jeter; sodelujejo v metabolizmu etanola.
- **Ksantinska oksidaza** - pretvarja hipoksantin v ksantin in potem v sečno kislino. Učinkovine kot substrati: teofilin, 6-merkaptopurin. Alopurinol je substrat in inhibitor ksantine oksidaze.

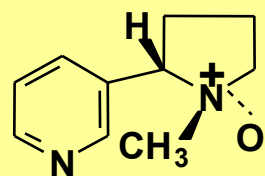
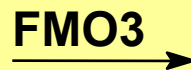
Ne-CYP oksidacija učinkovin - 2

- **Flavin monooksigenaze (FMO)**
 - Družina encimov, ki katalizira oksidacijo dušika, fosforja, žvepla; npr.: nastajanje N-oksidov
 - FMO izolirali iz jeter, pljuč (D. Ziegler, 1993, *Ann Rev Pharmacol Toxicol* 33:179-199)
 - Potrebujejo molekularni kisik, NADPH, flavin adenozin dinukleotid (FAD)
 - FMO so encimi občutljivi na toploto, brez kovinskih ionov,
 - Na FMO vplivajo prehrana, učinkovine in spol.

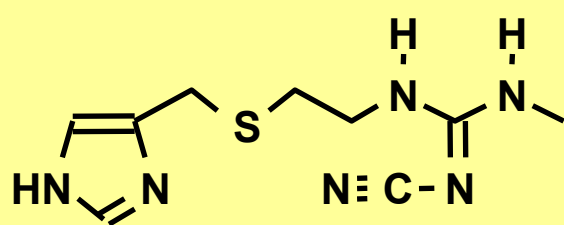
FMO Oksidacije



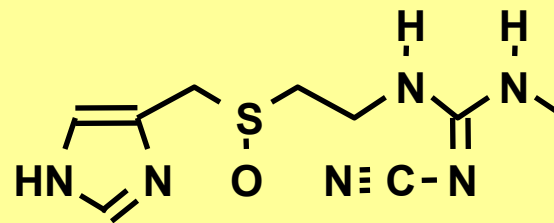
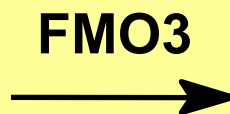
nikotin



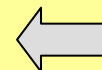
nikotin-N-oksidi



cimetidin

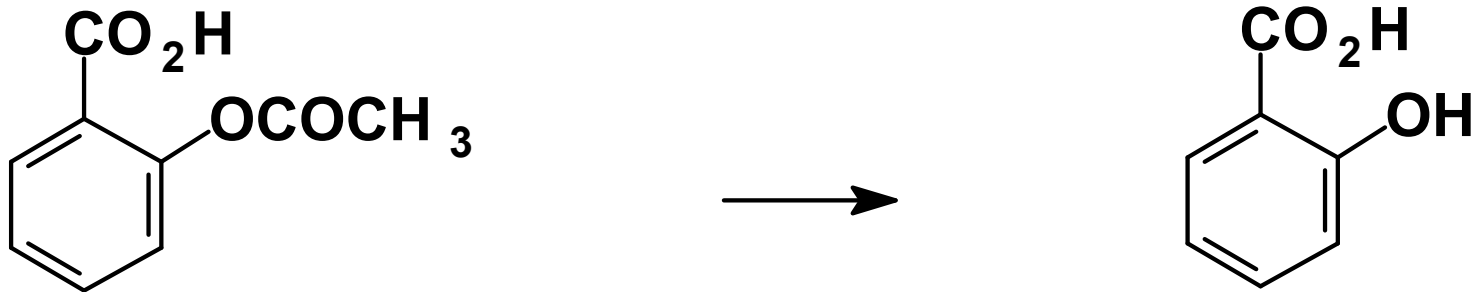
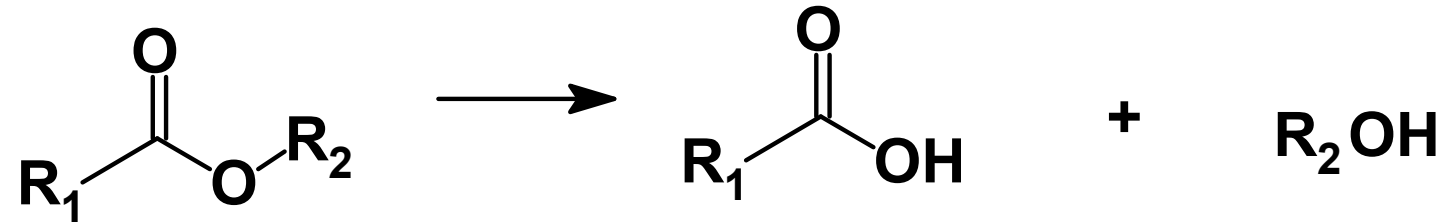


cimetidin S-oxid



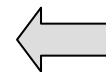
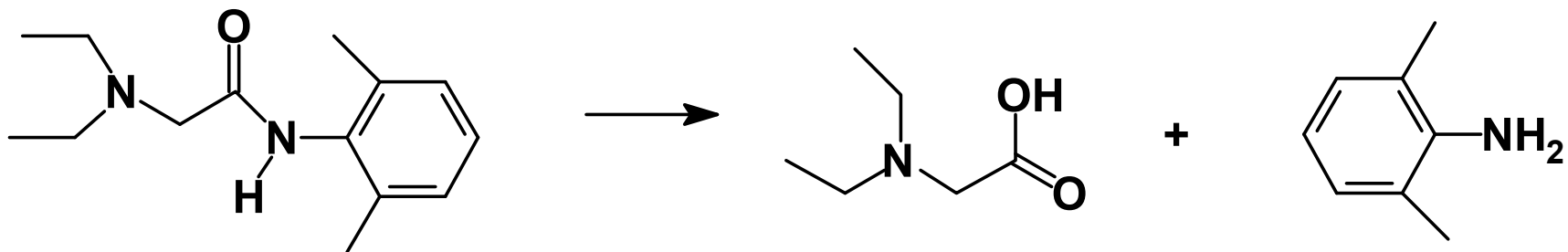
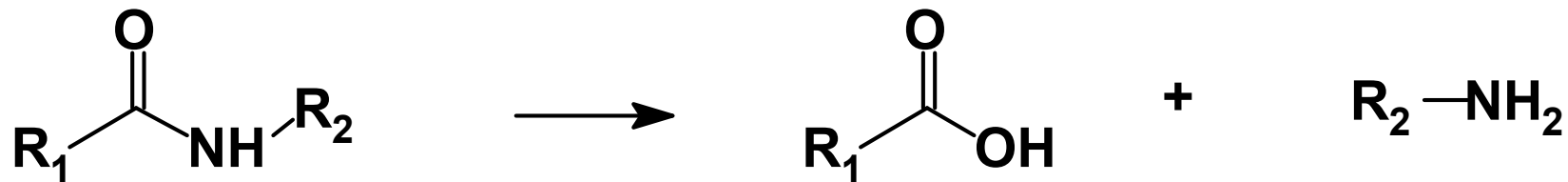
Hidrolize

Estri

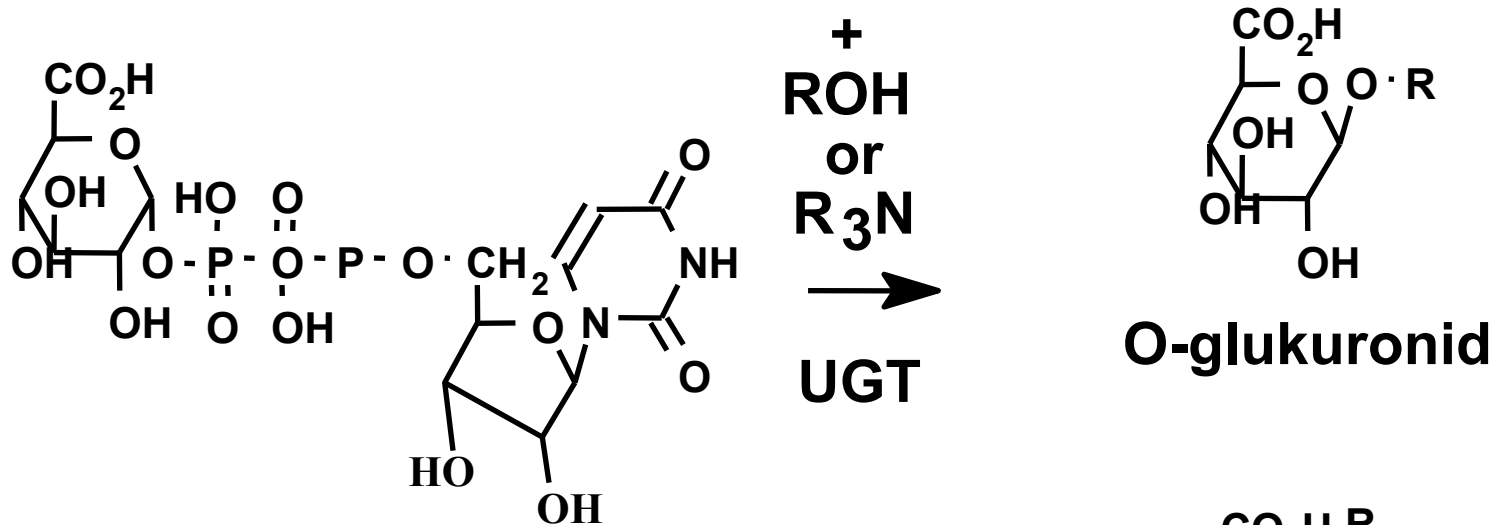


Hydrolize

Amidi

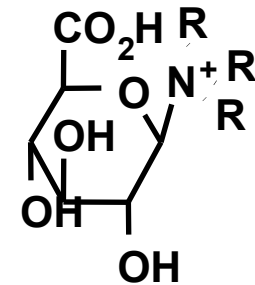


Konjugacije Glukuronidacija



UDP- α -D-glukuronska kislina

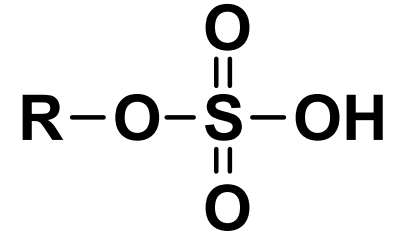
O-glukuronid



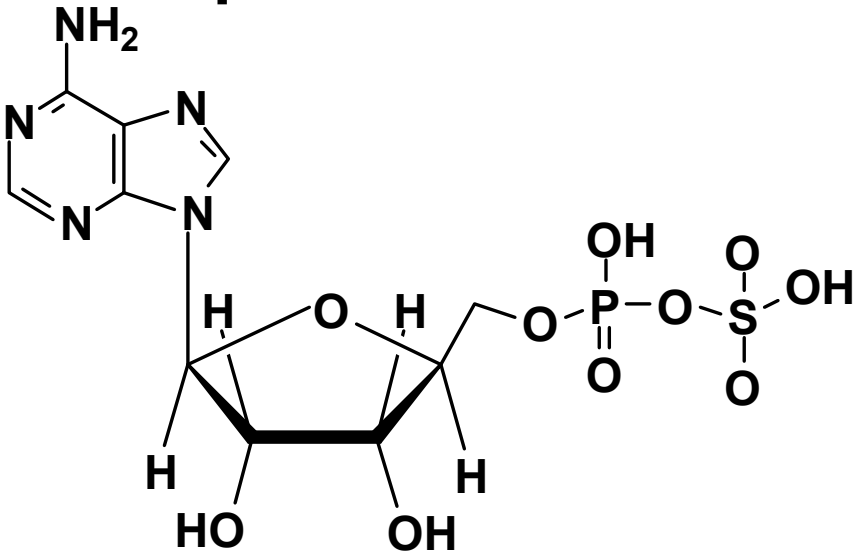
N⁺-glukuronid

V jetrih je več topnih UDP-Gluk-transferaz

Tvorba sulfatov

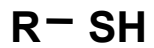
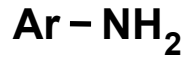


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(PAPS, 3'-fosfoadenozin-5'-fosfosulfate)

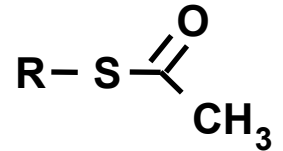
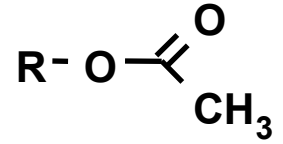
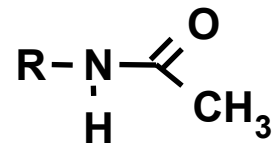
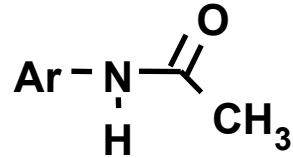
Acetiliranje



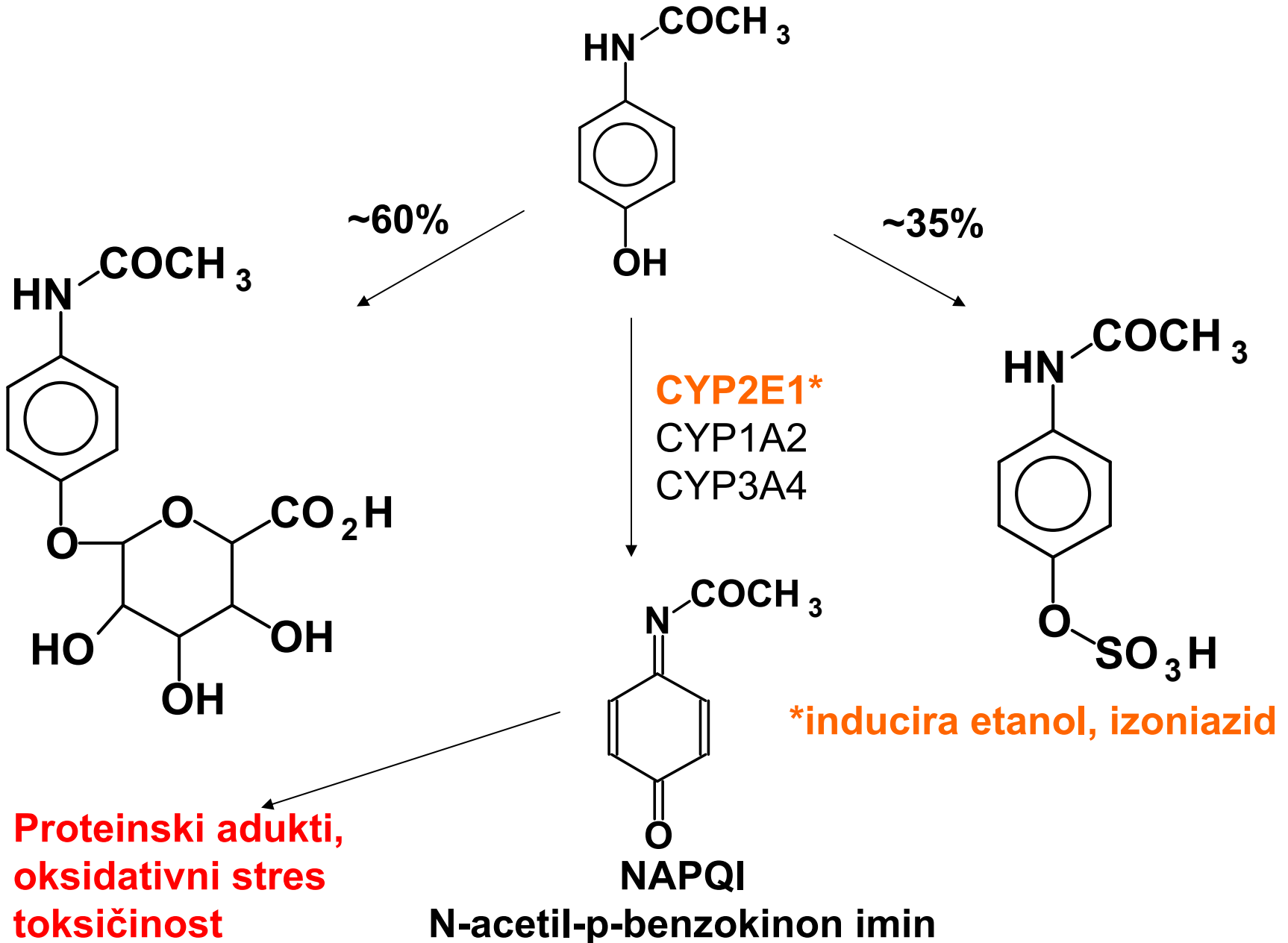
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Acetil transferaza



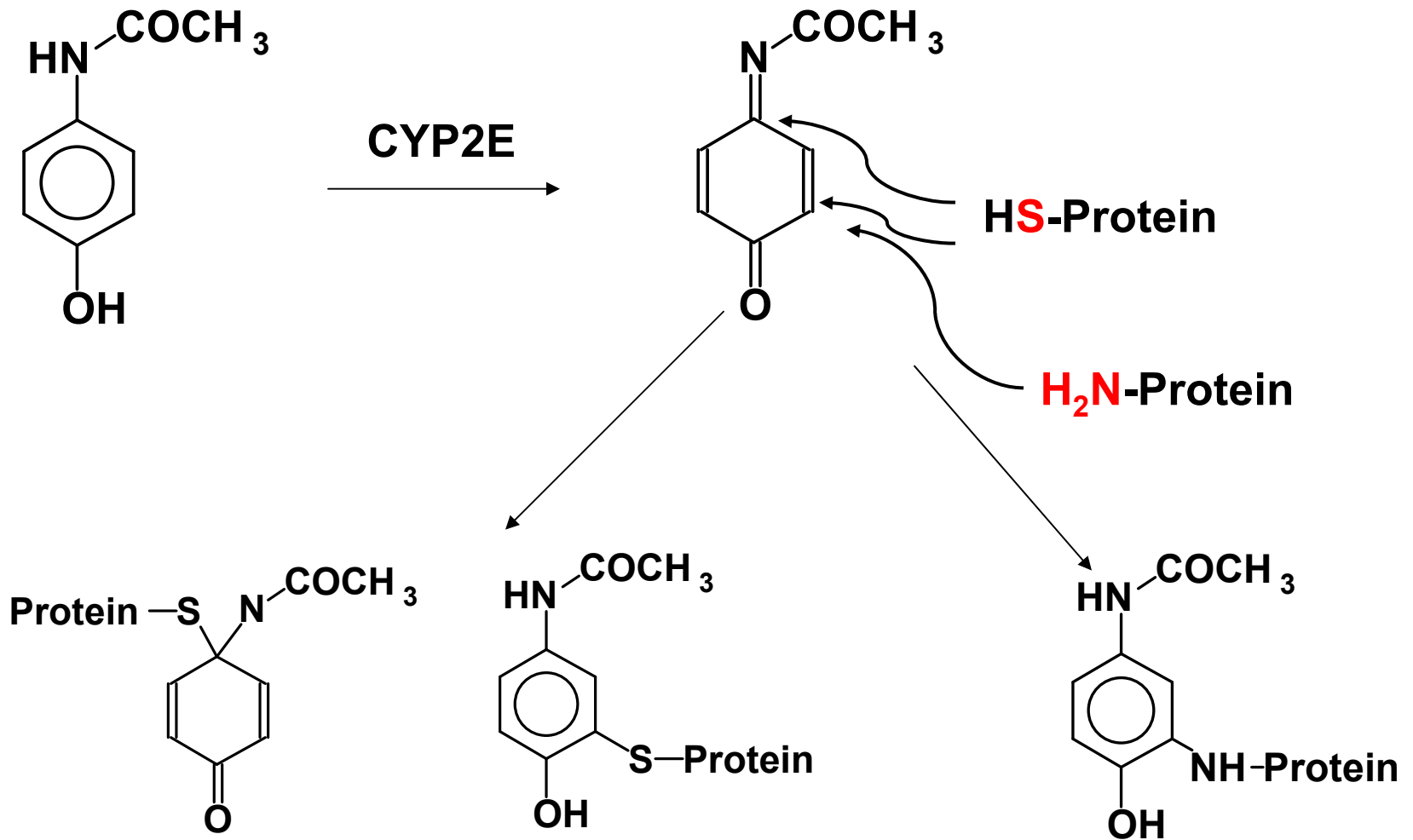
Metabolizem paracetamola



Toksičnost paracetamola

- Acetaminophen overdose results in more calls to poison control centers in the United States than overdose with any other pharmacologic substance.
- The American Liver Foundation reports that 35% of cases of severe liver failure are caused by acetaminophen poisoning which may require organ transplantation.
- *N*-acetyl cysteine is an effective antidote, especially if administered within 10 h of ingestion [NEJM 319:1557-1562, 1988]
- Addition of *N*-acetyl cysteine to acetaminophen tablets proposed to prevent liver toxicity. [British Medical Journal, Vol. 323, Sept. 15, 2001]

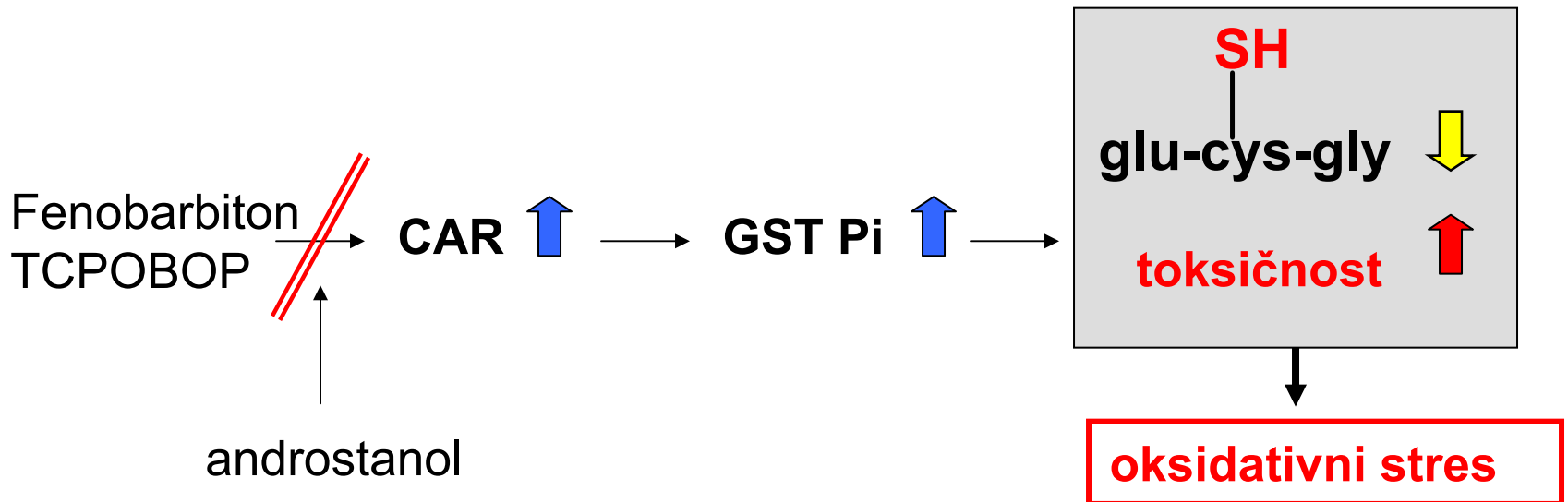
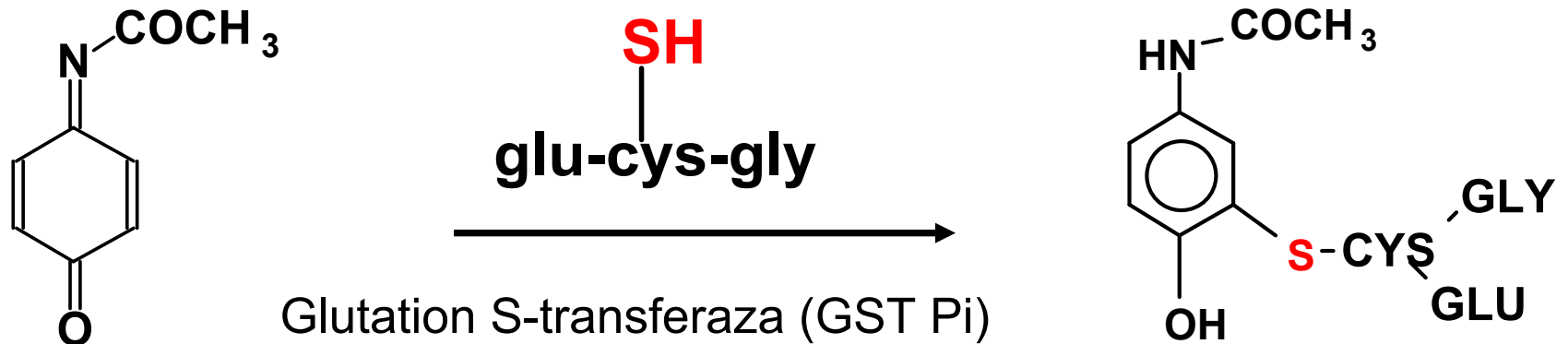
Adukti paracetamola s proteini



S.D. Nelson, *Drug Metab. Rev.* 27: 147-177 (1995)

J.L. Holtzman, *Drug Metab. Rev.* 27: 277-297 (1995)

NAPQI toksičnost povezana s CAR aktivacijo, in pomanjkanjem GSH



Drug Metabolism - WWW Information Resources

• <http://www.icgeb.trieste.it/p450/>

- Directory of P450 Containing Systems; comprehensive web site regarding all aspects of chemical structure (sequence and 3D) of P450 proteins from all species; steroid ligands; links to related sites including leading researchers on P450

• <http://www.panvera.com/tech/dmeguide/index.html>

- Drug Metabolism Resource Guide - catalog with useful information and characteristics of natural and recombinant drug metabolizing enzymes; assay methods

• <http://www.netsci.org/Science/Special/feature06.html>

- Site contains essay “The emerging role of ADME in optimizing drug discovery and design” RJ Guttendorf, Parke-Davis

• <http://www.fda.gov/cder/guidance/>

- Site contains many useful documents regarding drug metabolism and FDA recommendations including "Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies in Vitro", FDA Guidance for Industry.

Acetaminophen toxicity mechanism

- Mice nulled for glutathione S-transferase are resistant to acetaminophen toxicity
 - equal amounts of acetaminophen protein adducts formed in null and wild type suggesting protein adducts may not be toxic
 - hepatic GSH lowered in wild type (but not in KO) after acetaminophen
- CAR nulled mice are also resistant to acetaminophen toxicity
 - hepatic GSH lowered in wild type (but not in KO) after acetaminophen
 - CAR-humanized mice demonstrate same toxicity response
- N-acetyl cysteine is an effective agent to block GSH depletion and rescue from liver damaging toxicity
- NAPQI-protein adduction or NAPQI-GSH depletion-oxidative stress....to be continued