Module 5

# Drug Development and Metabolism

5_1 Drug development

5_2 Drug metabolism

5_3 Rational design of drugs

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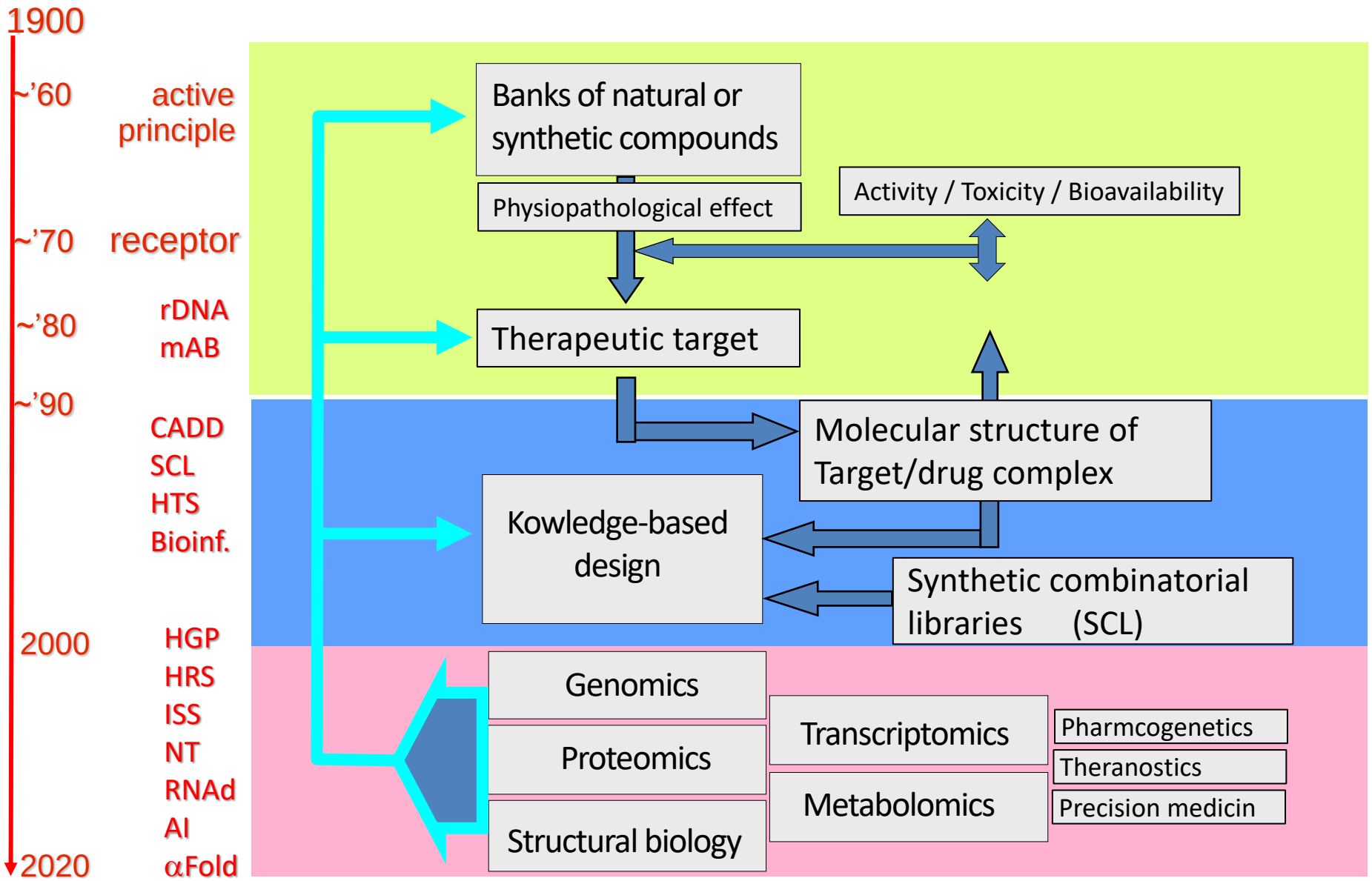
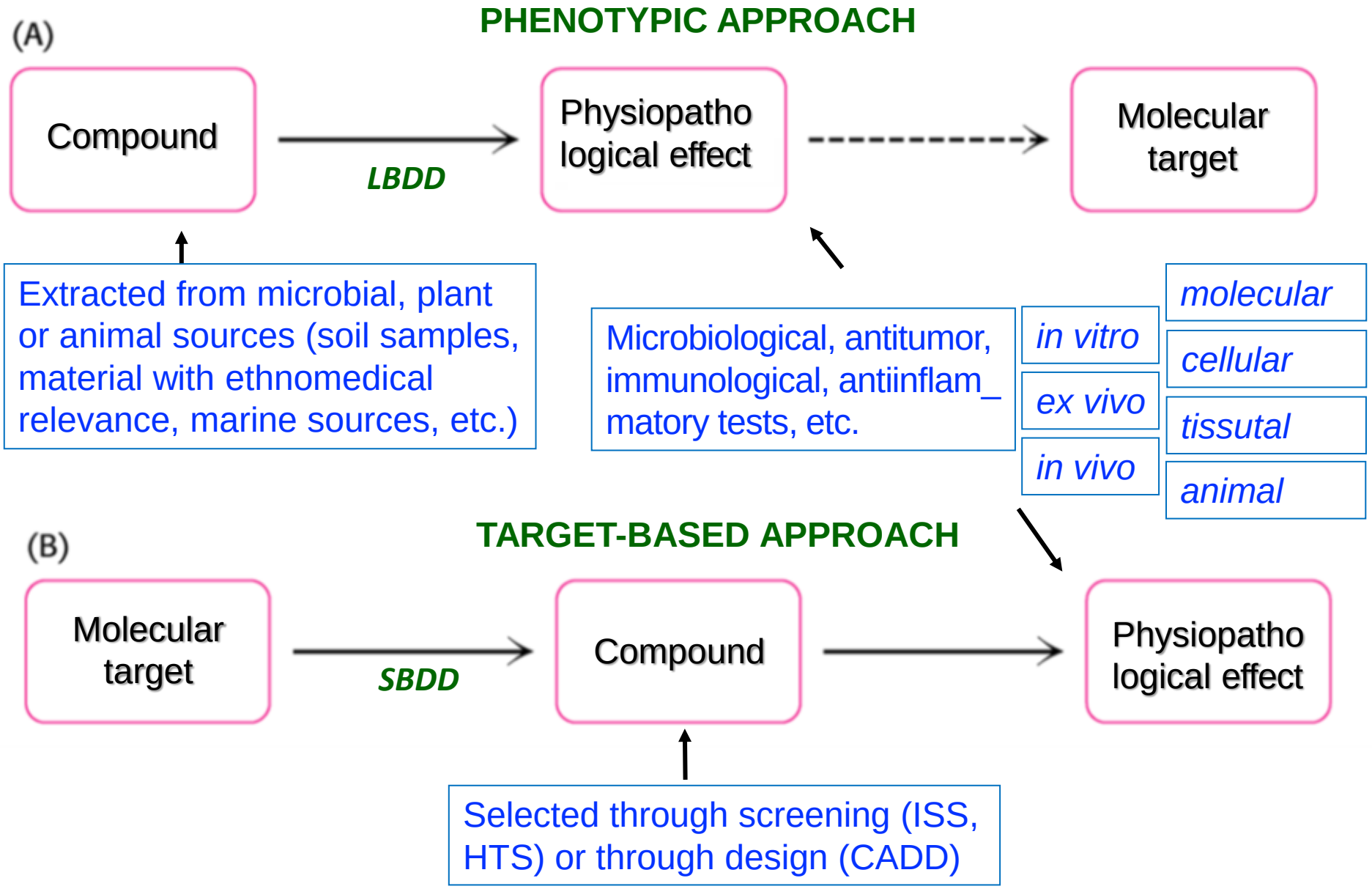
# EVOLUTION IN APPROACHES TO DRUG DISCOVERY



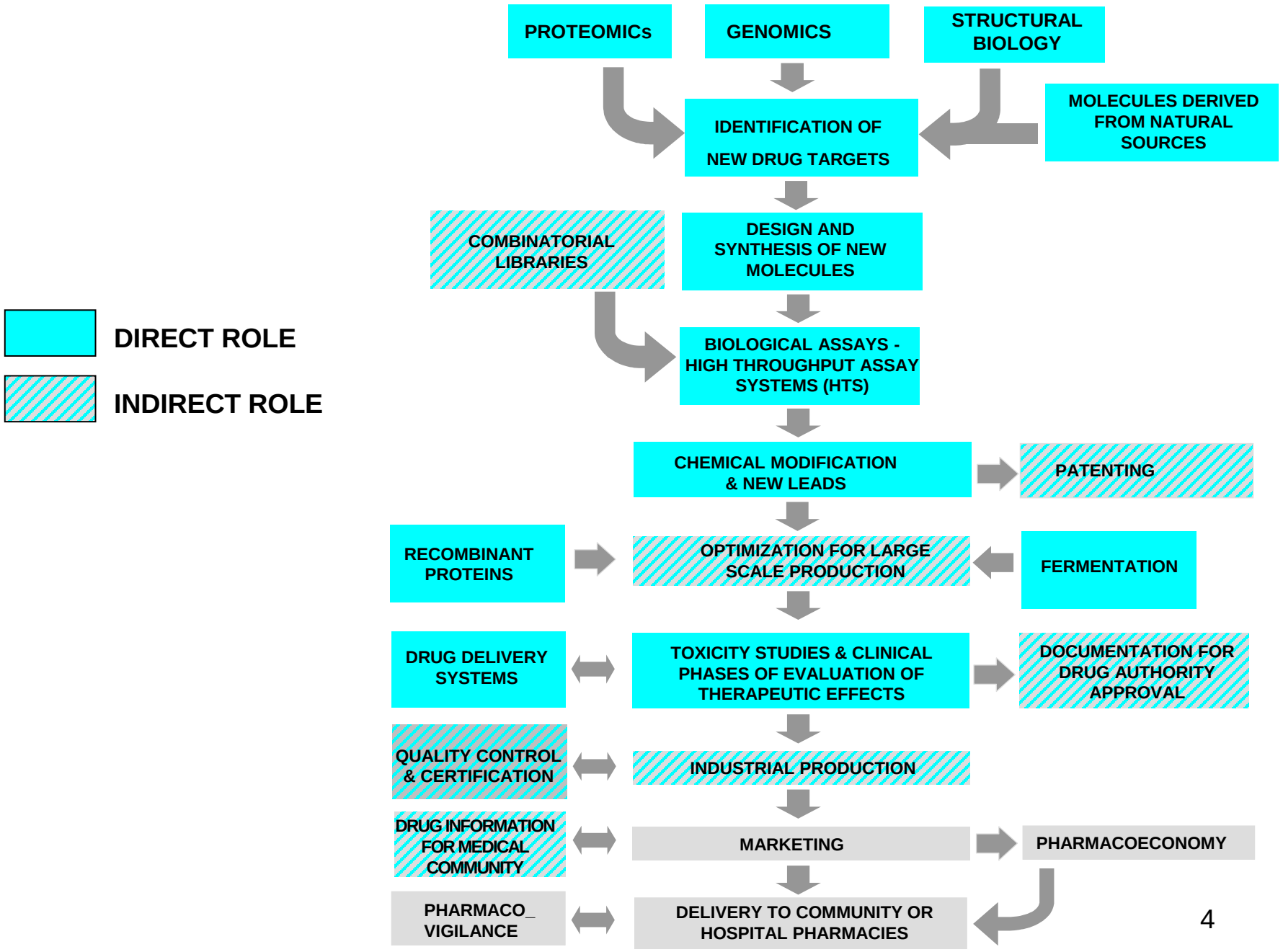

rDNA = recomb. DNA; mAB = monolonalAB; CADD = Computer Aided Drug Design; HTS = high trhoughput screening
SCL = Synth. Comb. Library; HGP = Human genome project; HRS = high resol. struct; ISS = *in silico* screening
NT = nanotecnoligies; RNAd = RNA drugs AI = Artificial intelligence αFold = Structure prediction algorithm

# EXPERIMENTAL APPROACHES IN DRUG DISCOVERY

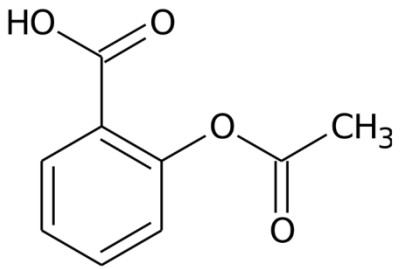



***Ligand Based Drug Design*** ***Structure Based Drug Design***

# ROLE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY IN DRUG DEVELOPMENT



## an early case: acetylsalycilic acid (aspirin)



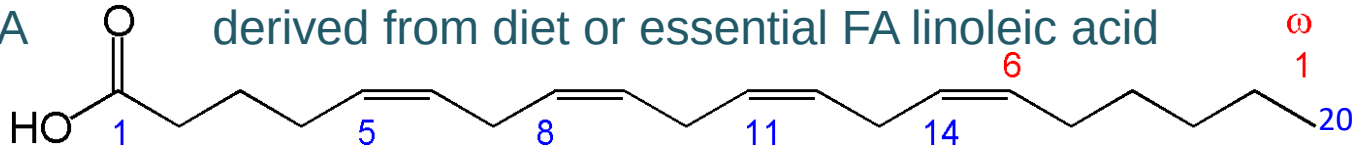

- Non Steriod Antiinflammatory Drug (NSAID) (*FANS in Italian*)
- ~ 40,000 tons (50 -120 billion pills) / year
- The effects of salycilate-rich plants (e.g. Salix) other has been known for thousands of years (ancient Sumers and Egyptians, Hyppocrates)
- Salicylic acid was known to have antipyretic, analgesic and anti-inflammatory properties
- In 1899 the drug company BAYER begins to produce synthetic «aspirin» - addition of the acetyl group makes it less irritating.
- Mechanism of action only understood in late 60s-70s. Supresses synthesis of prostagalandins & thromboxanes (mediate pain, inflmmation and fever)
- Its original role has declined, with newer drugs such a paracetamol and ibuprofen, but has found a new role as anti-clotting and heart drug.

Spirea ulmaria
(*Filipendula ulmaria*)

Salix alba
(salice bianco)

## Short-range signal molecules produced from arachidonic acid

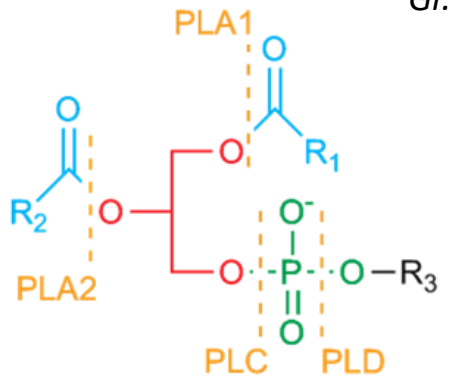
- Polyunsaturated LFA 20:4Δ5,8,11,14
  20:4 ω-6

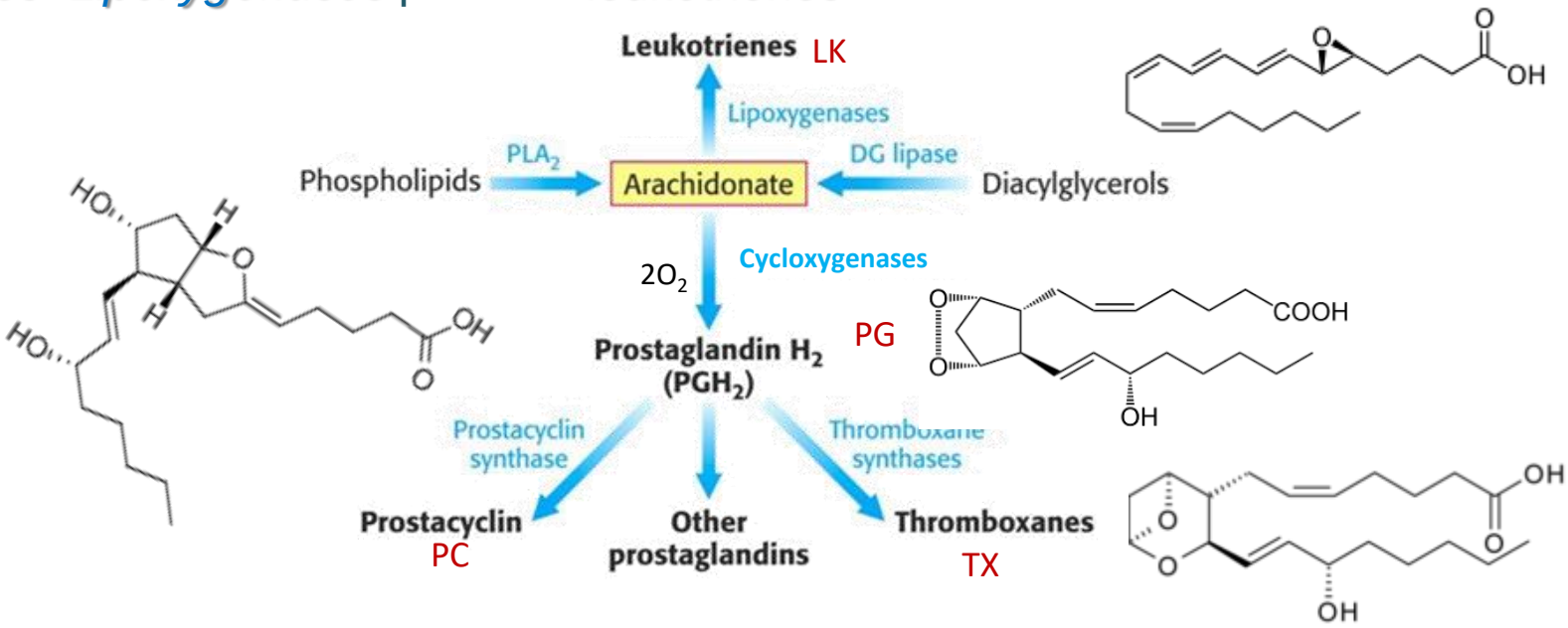



derived from diet or essential FA linoleic acid

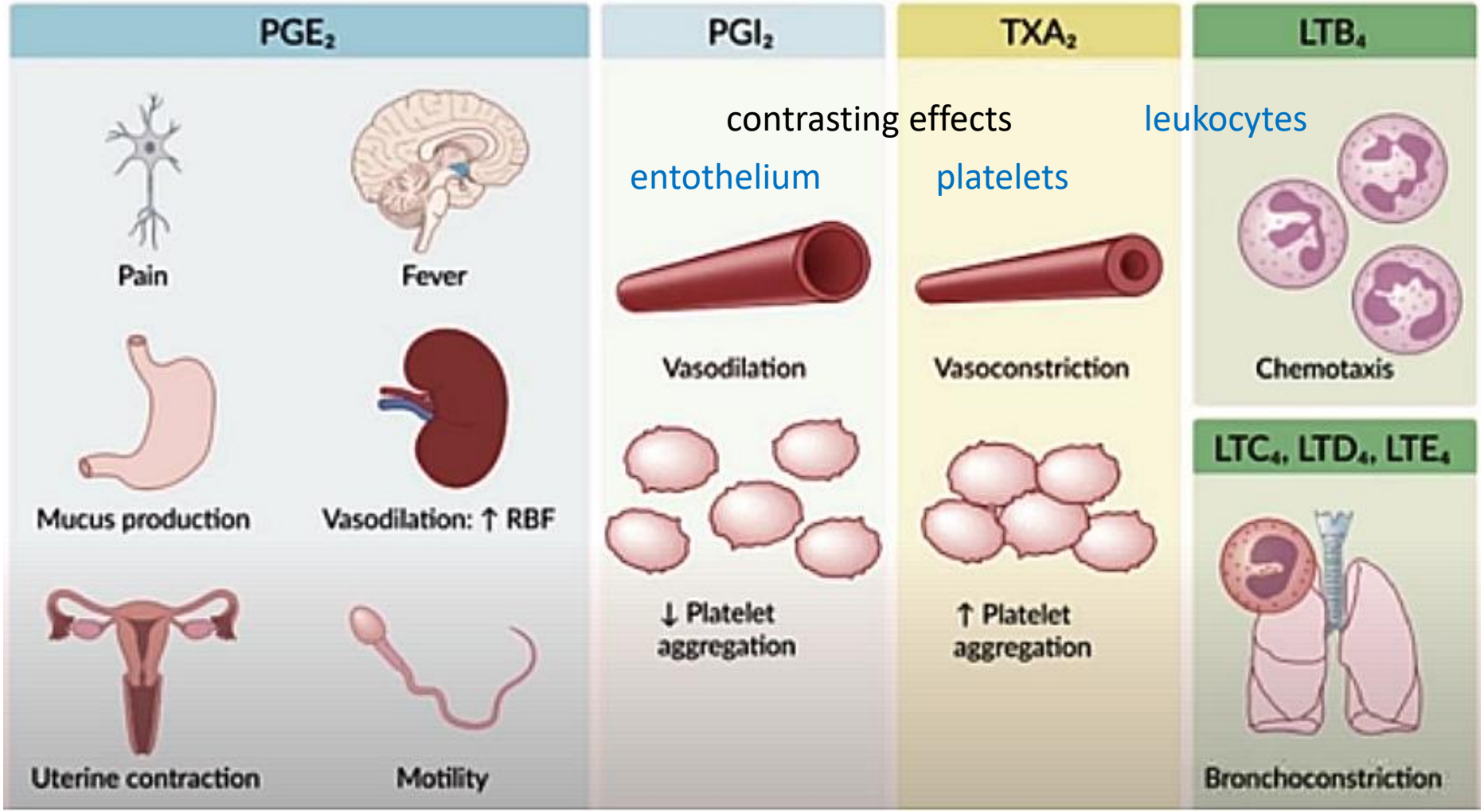
*Gr. Eicos*

- It is released from membrane phospholipids by *Phospholipase A2* (*PLA2*) or DAG (*DAG lipase*).
- This PUFA (Poly Unsat. FA) is then converted into eicosanoids by enzymatic or non-enzymatic oxidation (20 carbons and 3-5 double bonds are required).
- *Cyclooxygenases* (*COX*) produce prostaglandins and thromboxanes. *Lipoxygenases* produce leukotrienes.



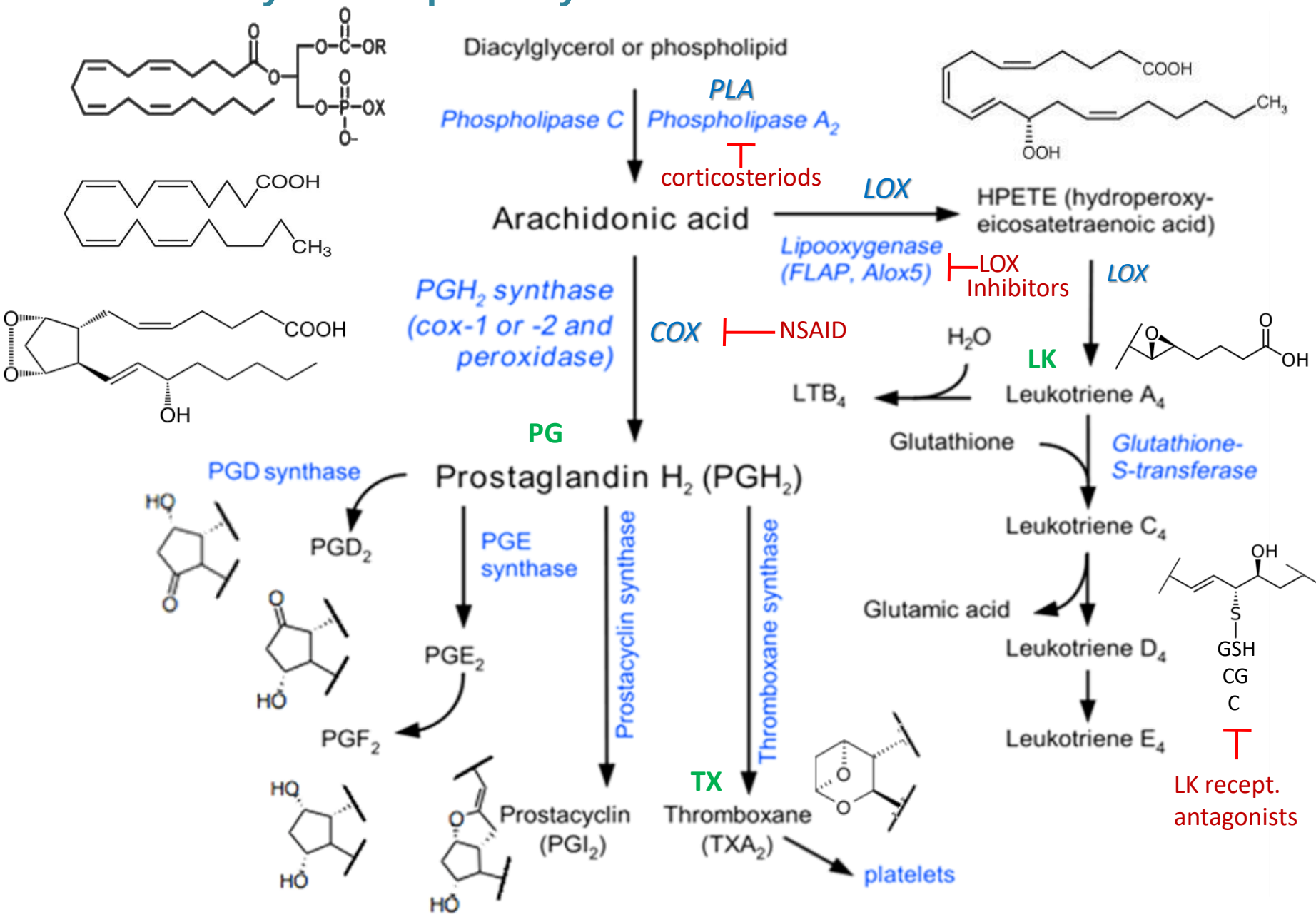
# FUNCTIONS OF EICOSANOIDS




- Eicosanoids function in various physiological contexts and pathological processes. They are autocrine or paracrine signals (short-range unlike hormones) that act on GPCRs.
- Their multiple functions underscore the importance of using drugs to control production or action of eicosanoids.

# EICOSANOIDS BIOSYNTHESIS

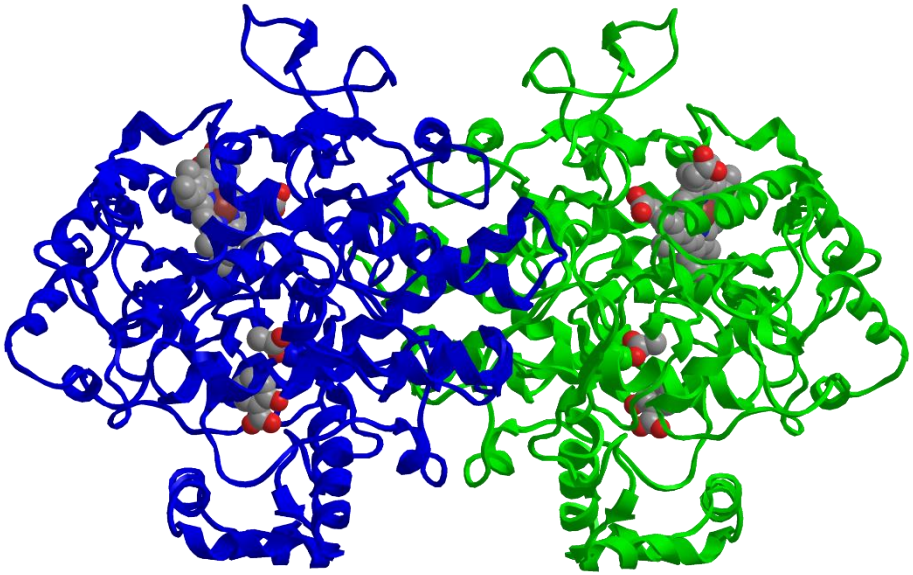
## Eicosanoid synthesis pathways from arachidonic acid



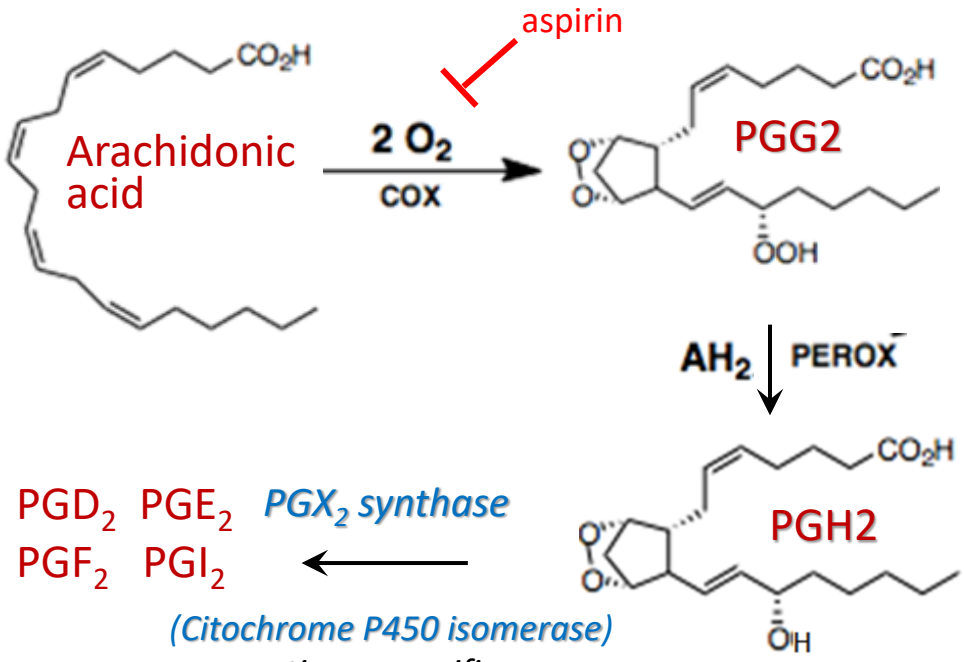
# TARGETS FOR ACETYLSALYCILATE

## COX-1 and -2

- *Prostaglandin endoperoxide synthases* (*PTGS*) are homodimeric enzymes with two activities: *cyclooxygenase* and *peroxidase*
- *COX-1* (constitutive) produces PG in physiological processes (e.g., gastric mucosal protection, platelet aggregation); *COX-2* (induced) participates in inflammatory reactions
- They convert arachidonic acid into prostaglandin H2 (PGH2).
- Both inactivated by aspirin; acetylates a serine residue in arachidonic acid binding site (a form of suicide inhibition)
- Aspirin inhibits *cyclooxygenase* activity but not *peroxidase* activity.

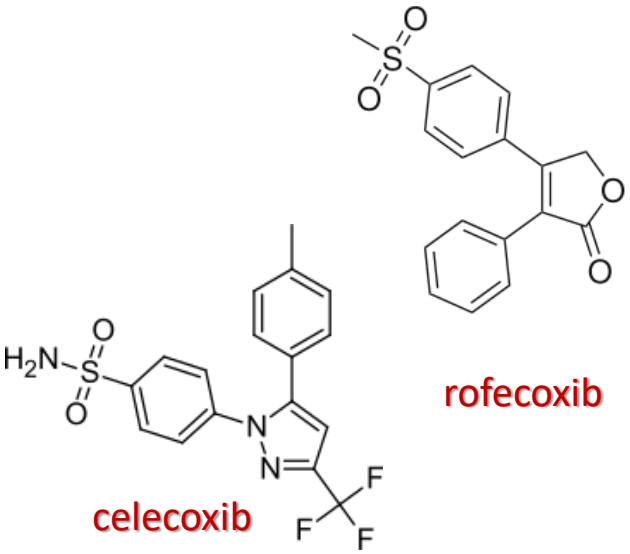


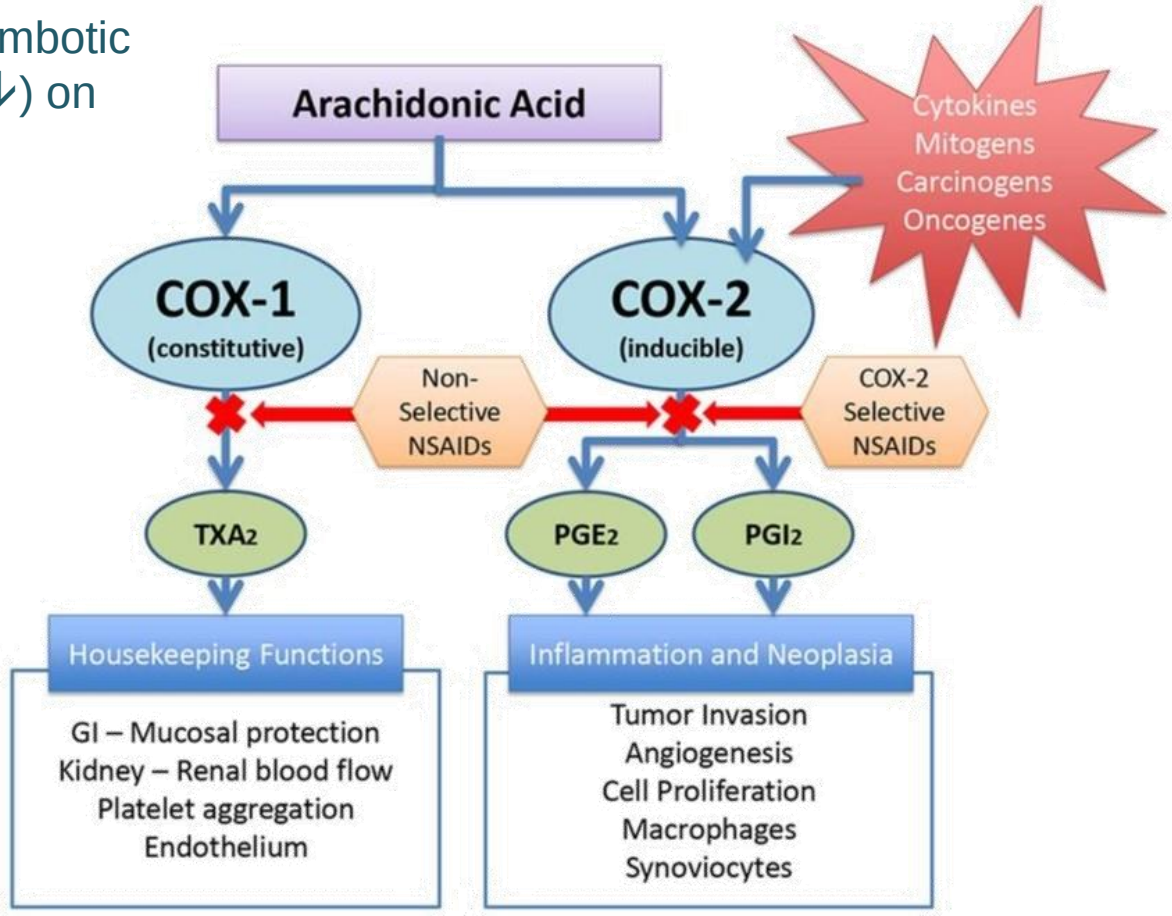
*Structure of COX-2 inactivated by Aspirin.*



## Inibition of *Cox-2*

- Inhibiting PG production can have beneficial effects but also side effects.
- Inhibiting *COX-2* (induced) reduces fever and inflammation; inhibiting *COX-1* (constitutive) reduces its protective role in gastrointestinal mucosa and tissue.
- More specific *COX-2* inhibitors [late 1990s, celecoxib, rofecoxib (withdrawn), meloxicam] act without damaging the gastric mucosa.
- At low concentrations, the antithrombotic action of aspirin (anti*COX-1*, TX ↓) on platelets is beneficial.
- Conversely, COX-2 inhibitors can have cardiotoxic effects (PGI2 ↓).

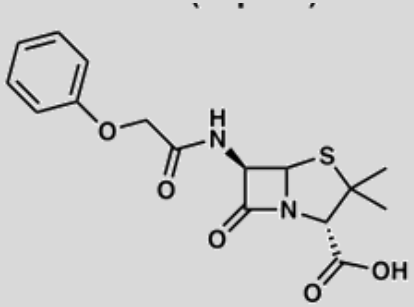


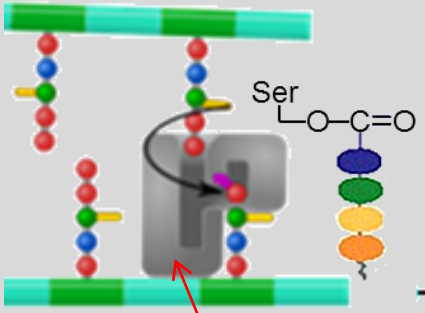
## Other natural sources for drugs

| SOURCE | MOLECULE | TARGET | EFFECT |
|---|---|---|---|

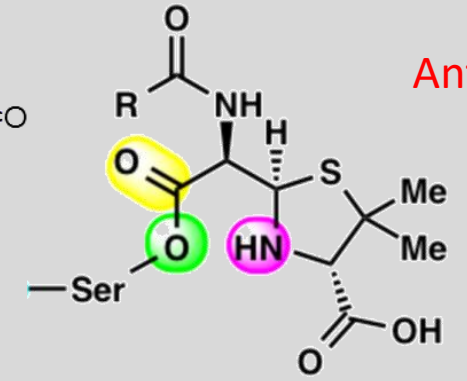
*Penicillium chrysogenum*




penicillin




peptidoglycan *transpeptidase*

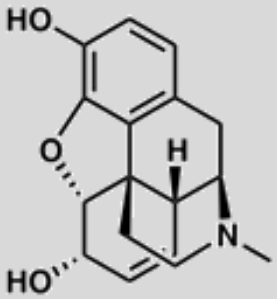



Antibiotic

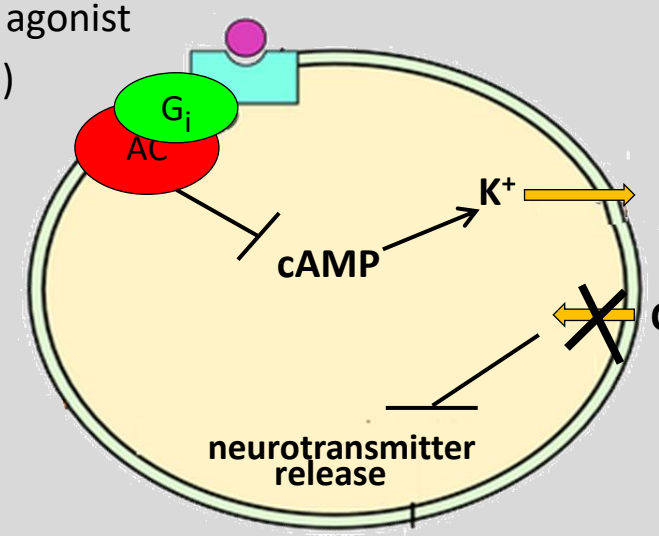
*Papaver somniferum*

opioid receptor agonist

(GPCR with $G_{\alpha i}$ )




morphine




( μ–opioid receptor, MOR, GPCR )

Analgesic

# CASE STUDIES FOR DRUG DEVELOPMENT – NATURAL SOURCES

## Other natural sources for drugs

| SOURCE | MOLECULE | TARGET | EFFECT |
|---|---|---|---|

*Bothrops jararaca*

**Pyr**-WPRPQIP**P**
teprotide

captopril

Angiotensin I → (*Angiotensin Converting Enzime* (*ACE*)) → Angiotensin II

*ACE inhibitor*

Angiotensin II → ATR 1,2 (GPCR) → aldosterone → *vasoconstriction* *blood volume* ↑

Inactive fragment3 ← Bradykinin ← $B_2$ recept. (GPCR) ← $NO, PGI_2$ ⟹ *vasodilation*

Antihyper_
tensive

*Penicillium chrysogenum* →

*Aspergillus terreus*

*Pleurotus spp.*

mevastatin

lovastatin

Synthetic → atorvastatin

3-hydroxy-3-methylglutaryl-CoA
(HMG-CoA)

STATINS
(HMG-Coa Inhibitors) ⊣ *HMG-CoA reductase*

Mevalonic acid

cholesterol

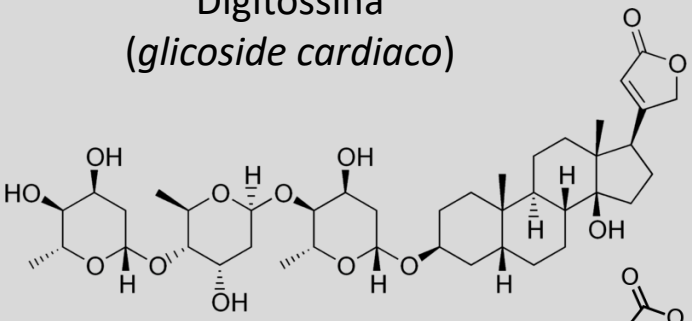
Anticholesterol

## Other natural sources for drugs

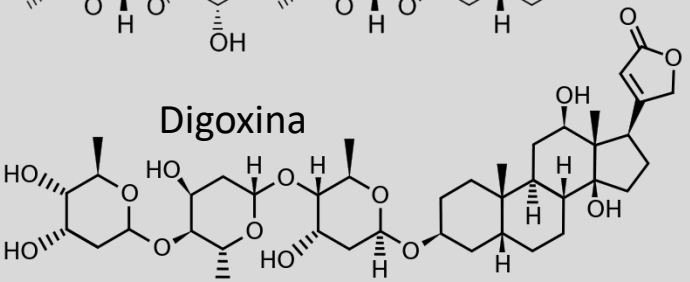
| SOURCE | MOLECULE | TARGET | EFFECT |
|---|---|---|---|

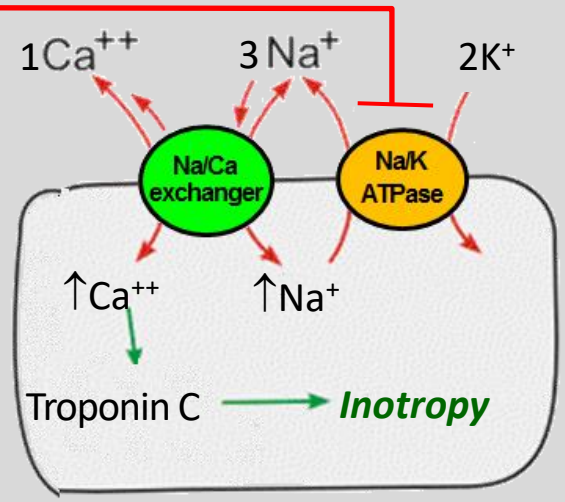
*Digitalis purpurea*

Digitossina
(*glicoside cardiaco*)



Digoxina

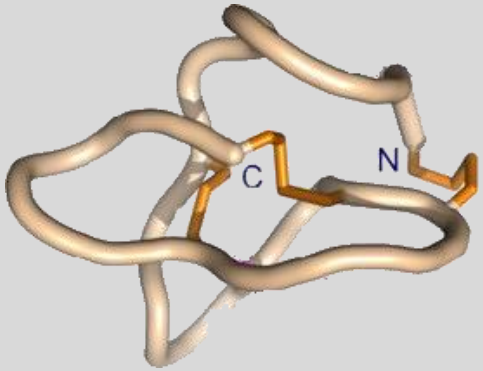




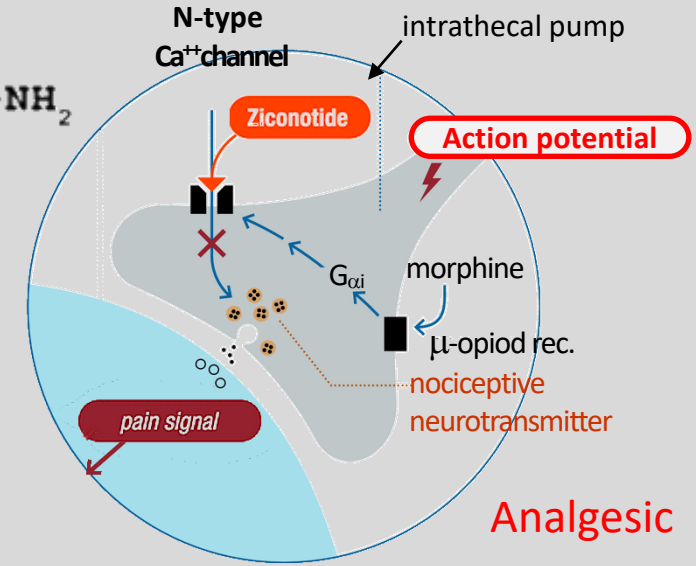

Inotropic

Cardiac muscle

*Conus magus*


Ziconotide (ω-conotoxin)



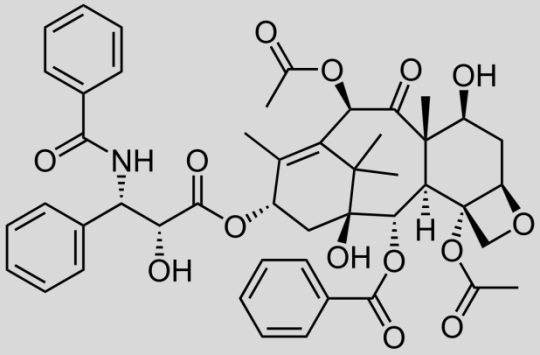

Analgesic

# CASE STUDIES FOR DRUG DEVELOPMENT – NATURAL SOURCES

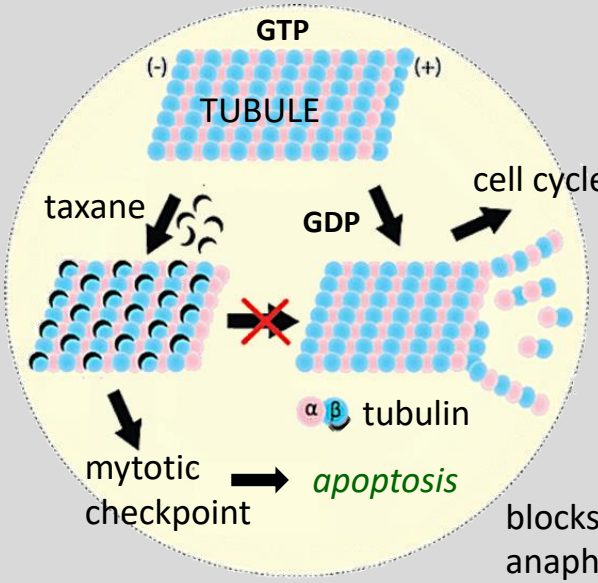
## Other natural sources for drugs

| SOURCE | MOLECULE | TARGET | EFFECT |
|---|---|---|---|

*Taxus brevifolia*



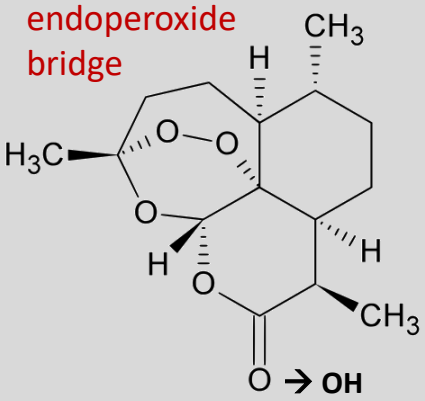
Paclitaxel
(*tetracyclic diterpene - taxane*)



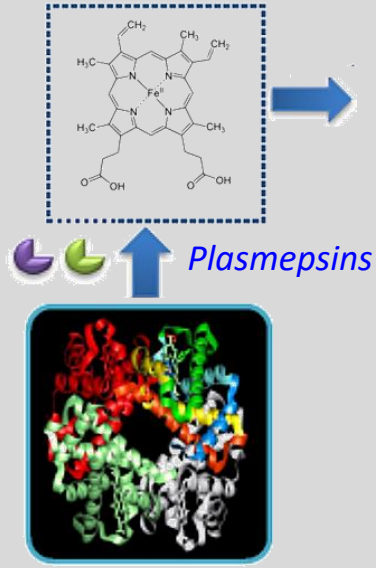

Anticancer

blocks the metaphase-anaphase transition

*Artemisia annua*



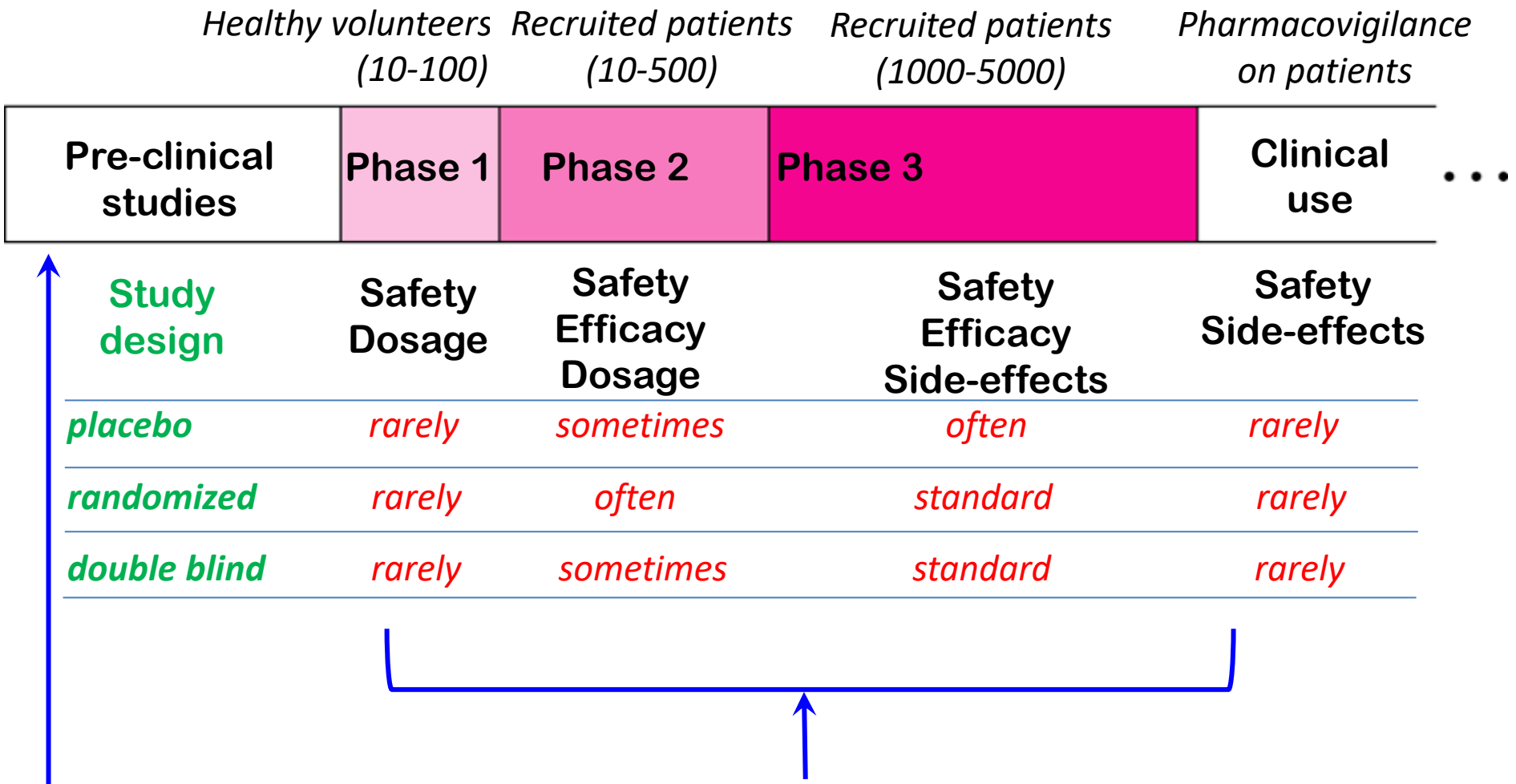

Artemisinin → Artenimol
(*tetracyclic diterpene - taxane*)




$Fe^{++}$ + artemisin
↓
ROS

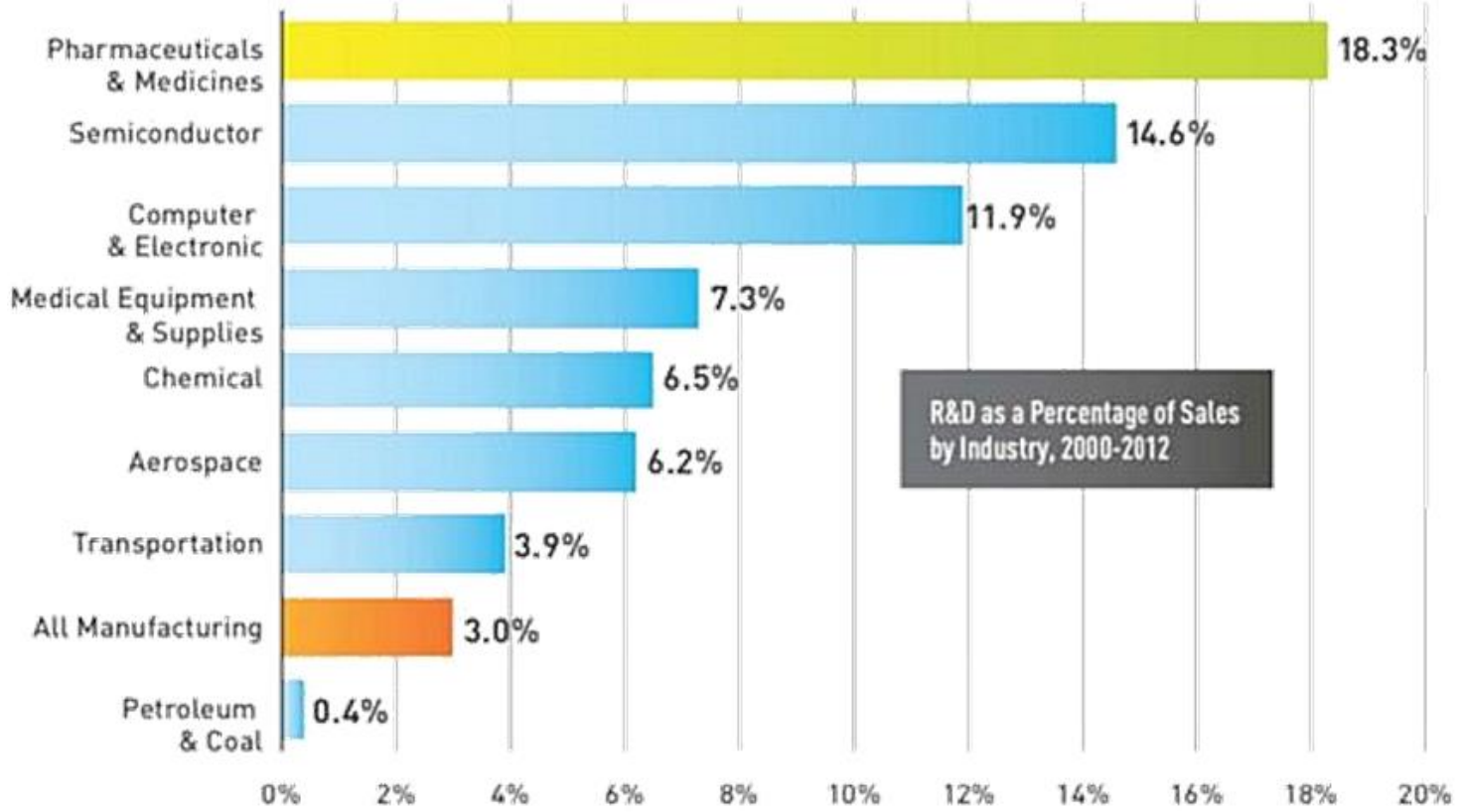
Antimalarial

# DRUG DEVELOPMENT PHASES



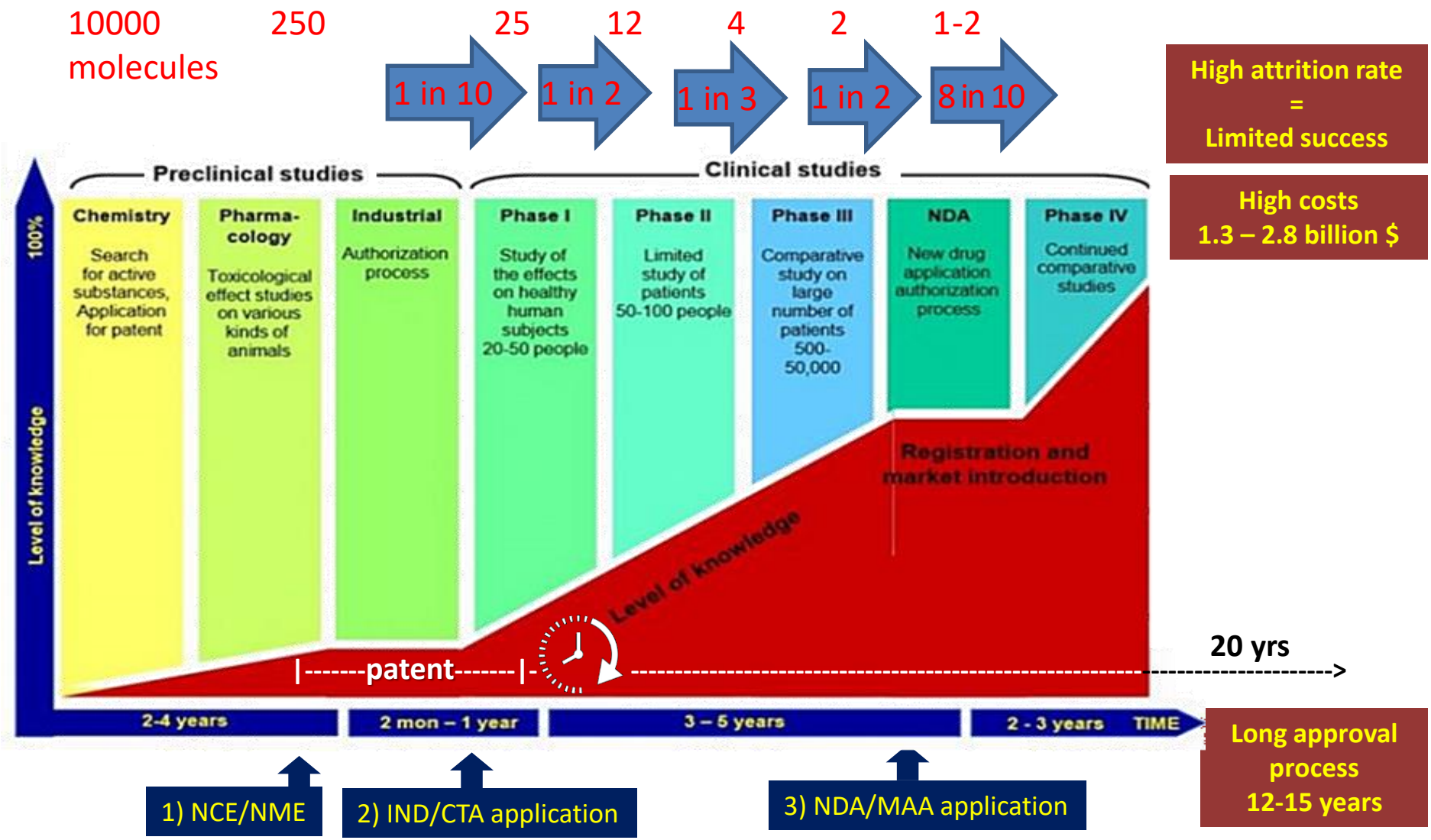
# DRUG DEVELOPMENT COSTS

## R&D as a percentage of sales by industry (2000-2012)



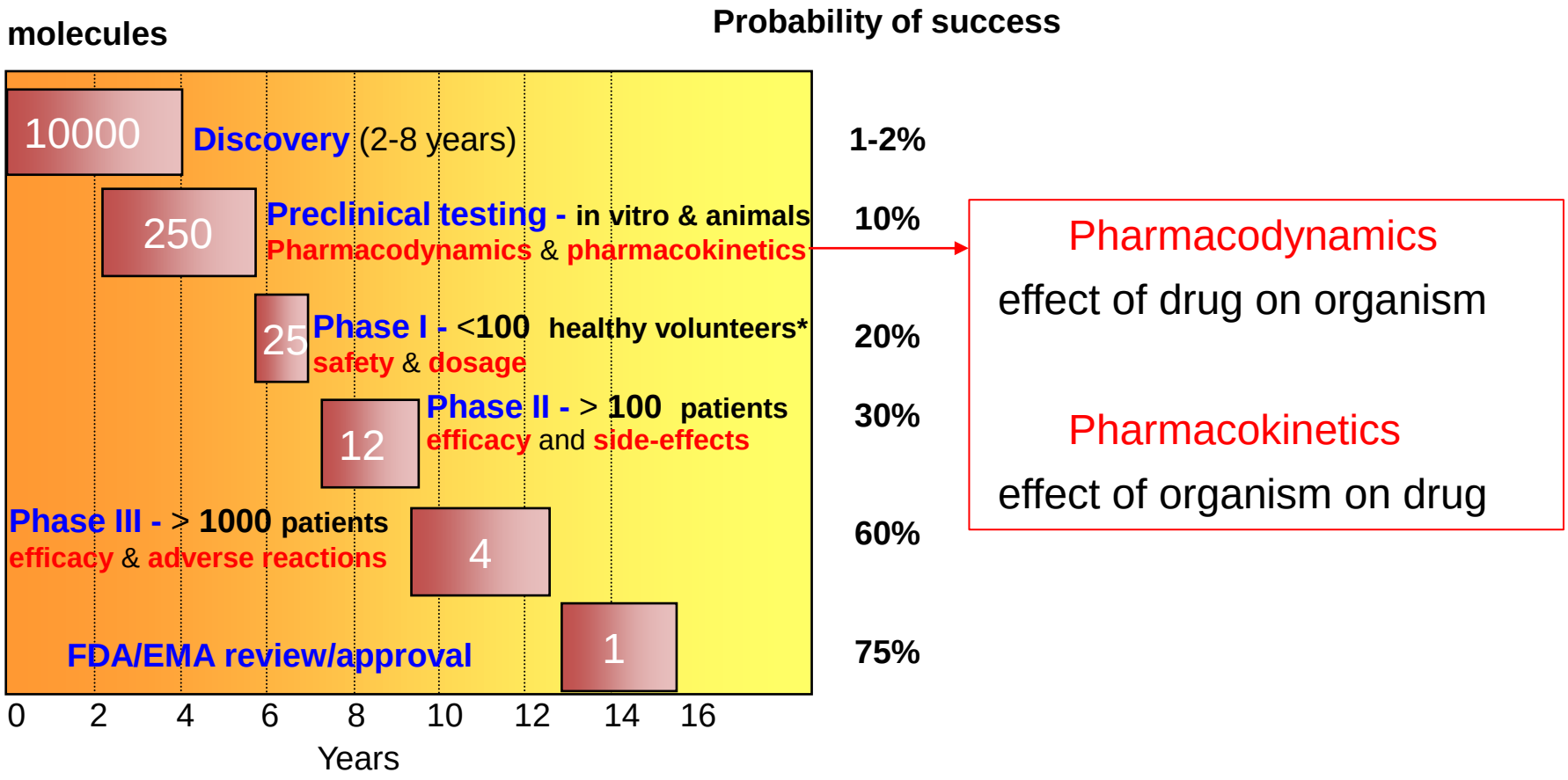

- R&D costs for drug development higher than for most other industrial sectors
- Apart from the «Discovery» costs (basic and applied reearch), costs for *Investigational New Drug* (*IND*) approval and for Preclinial studies and Phase I,II and II are very high
- The overall costs also reflect that most drug candidates fail at various stages. Development costs for a successful drug include «cost of failure» of all the others.

# DURATION & COSTS OF DRUG DEVELOPMENT PHASES




**Milestones**: 1) *New Chemical Entity / New Molecular Entity* declaration
2) *Investigational New Drug* (USA→FDA) or *Clinical Trial Application* (EU→EMA)
3) *New Drug Application* (USA→FDA) or *Marketing Authorization Application* (EU→EMA)

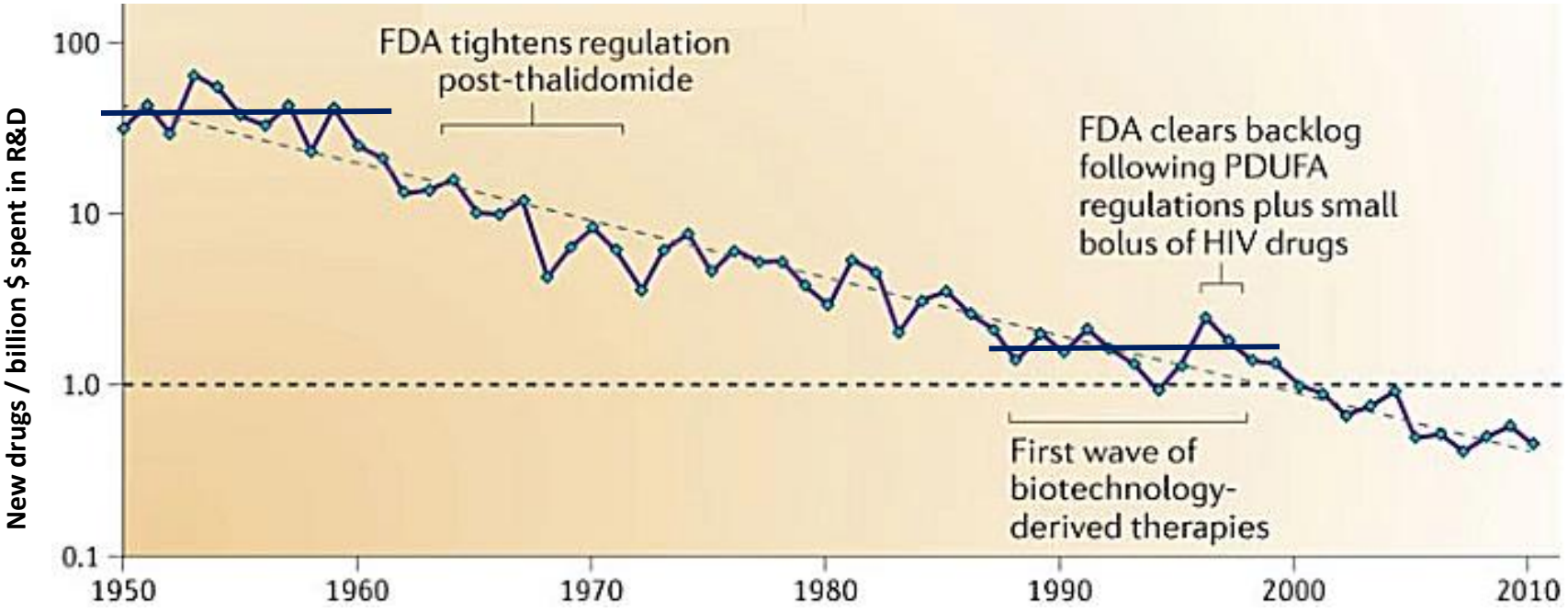
# SUCCESS PROBABILITY




- Phase I, II, and III clinical trials can take more than 7 years
- On average, it takes 12-15 years for a new drug to be approved
- Many molecules fail to pass the transition from *in vitro* to *in vivo* testing
- Few enter Phase I
- Success probability depends on drug type (all drugs ~15% anticancer < 5% vaccines >30%)

# CRITICAL SITUATION OF THE PHARMACEUTICAL SECTOR

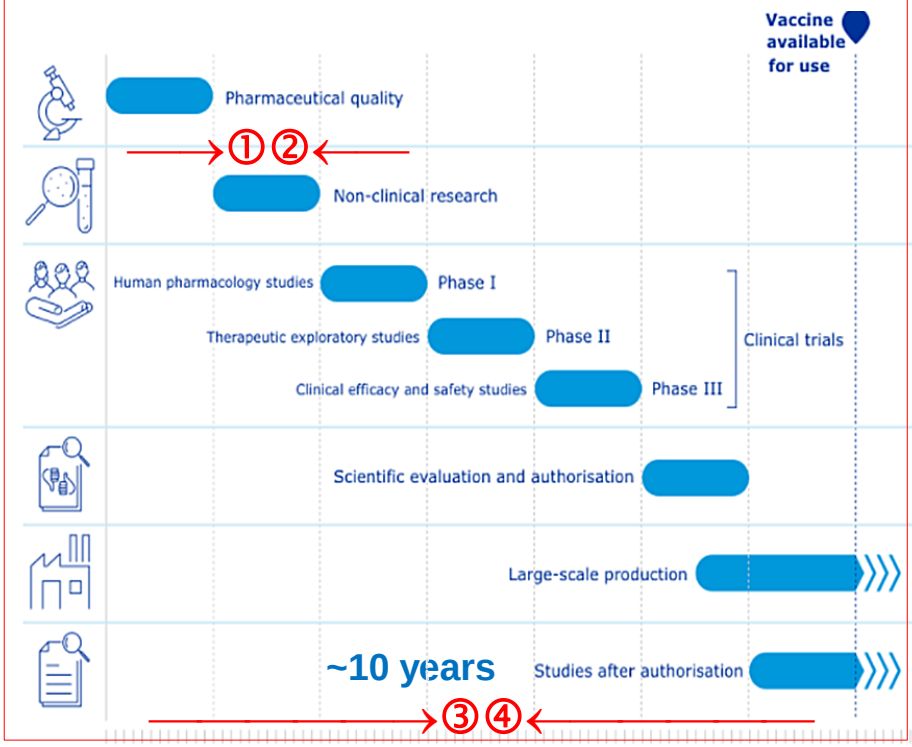
- The failure rate for drug approval is quantified as the **attrition rate**
- The attrition rate **has a significant impact on costs**
- **Failures generate >80% of R&D costs**, and must be recovered by approved drugs
- **R&D investments grows exponentially** - significant impact on company fixed costs
- High fixed costs → **continuous mergers**
- Despite continuous technological improvements **<1% research programs** identify products that **reach the market**
- In many cases the drug is unable to recoup the R&D investments
- The number of approved drugs per billion dollars spent continues to decline.



# REDUCING DEVELOPMENT TIMES – THE COVID VACCINE CASE

- **COVID-19 vaccines development shows how drug development efficiency can be significantly improved**

## Conventional drug development procedure




## Prcedure used for Covid-19 vaccines

- **January 2020:** Genetic sequence of SARS-CoV-2 published
- **Spring 2020:** Vaccine trials begin
- **December 2020**: EMA grants conditional MAA (CMA) for mRNA vaccine **Comirnaty** (BioNTech/ Pfizer)
- **January 2021:** EMA grants second CMA for the mRNA vaccine **Moderna**

**All stages of the drug development stages were respected**

Development benefited from:

① **previous studies** on other human SARS-CoV-2 related coronaviruses that caused SARS (Severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) epidemics

② **use of previous research** on messenger RNA (mRNA) technology;

③ **significant human and financial resources made available in a very short timeframe**;

④ use of **parallel phases** and ***rolling review***

## Use of previous studies and already carried out research

- **Main available platforms:**

- Inactivated virus vaccines: SARS-CoV-2 cultivated in cells and chemially inactivated
  *VeroCell – Sinovac (RollRev); VLA2001 – Valneva (Roll Rev)*

- Attenuated vaccines: genetically weakened versions that induce an immune response
  *COVI-VAC – Codagenix*

- Recombinant protein vaccines: *spike*, *receptor binding domain* (RBD) or *virus-like particle* (VLP).
  *Nuvaxovid – Novavax (MAA), Vidprevtyn – Sanofi (RollRev);*

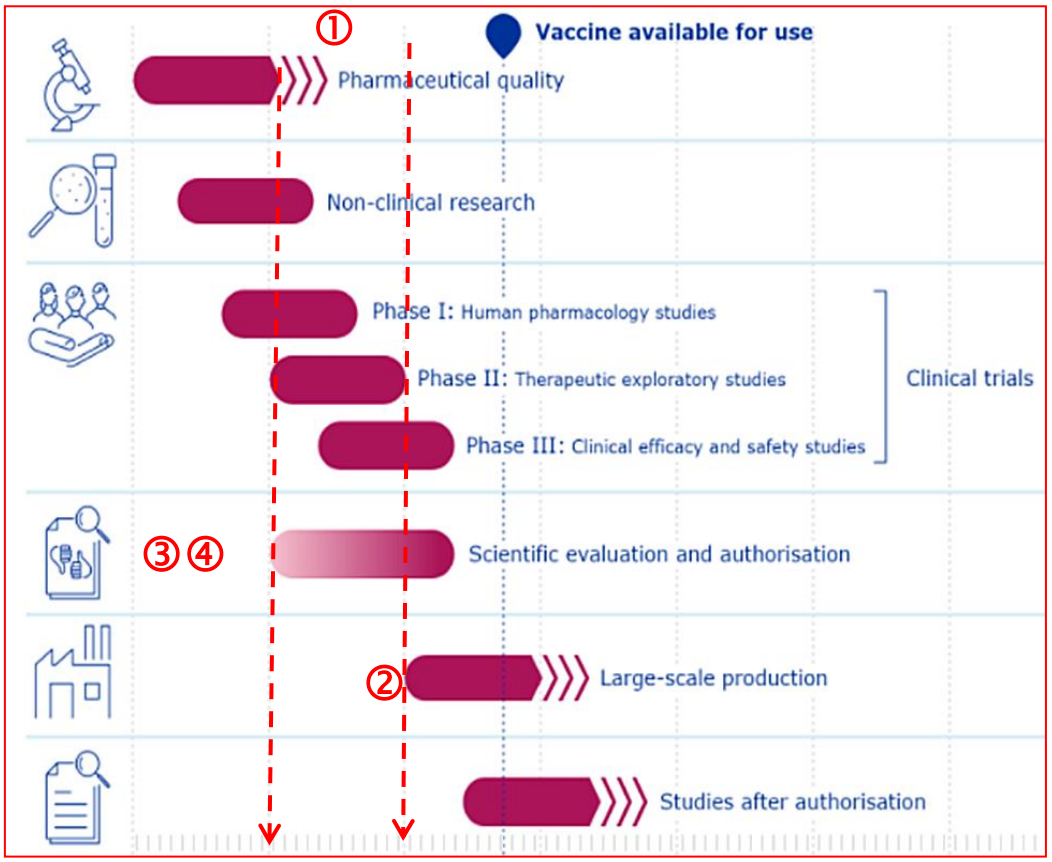
- DNA vaccines: plasmids that carry viral genes (es. for the *spike* proteins )
  *Zydus Cadila (approved in India)*

- Viral vector vaccines: incompetent viruses (eg. adenovirus) with genetic sequences for *spike proteins*
  other viruses *Vaxzevira – AstraZeneca (App.); Johnson & Johnson (App.); Sputnik (RollRev)*

- RNA vaccines: mRNA or autoreplicating RNA that codes for *spike proteins*.
  *Comirnaty – Pfizer/ BioNTech and SpikeVax – Moderna*

## Parralel phases, rolling review and increased efficiency

- The **rolling review** is a regulatory tool used by the EMA to **expedite the evaluation** of promising medicines or vaccines during a public health emergency.
- The EMA's **Human Medicines Committee** (**CHMP**) begins its evaluation based on experimental (preclinical) studies and preliminary clinical data; Reviews continuous updates




Parallel phases and *rolling review*

① Evaluation and study phases condocted as far as possibile in parallel, not in tandem

② Production of the vaccine initiates in parallel with a clinical phases and MAA

③ Bureaucratic/administrative process made more efficient

④ Rolling review – results evaluated by regulatory agencies when they are produce and not only after completion of the studies

## Biochemical studies for drug failure

- The high failure rate associated with drug development requires careful root cause analysis and ongoing decision-making to avoid very costly failures.
- Analysis of the major causes of failure in drug development provides us with their contribution to the attrition rate
- 40% of failures can be attributed to pharmacokinetic causes (absorption, distribution, metabolism, excretion) and 30% to toxicological causes.

- ABSORPTION – A
- DISTRIBUTION – D
- METABOLISM – M
- ECRETION – E

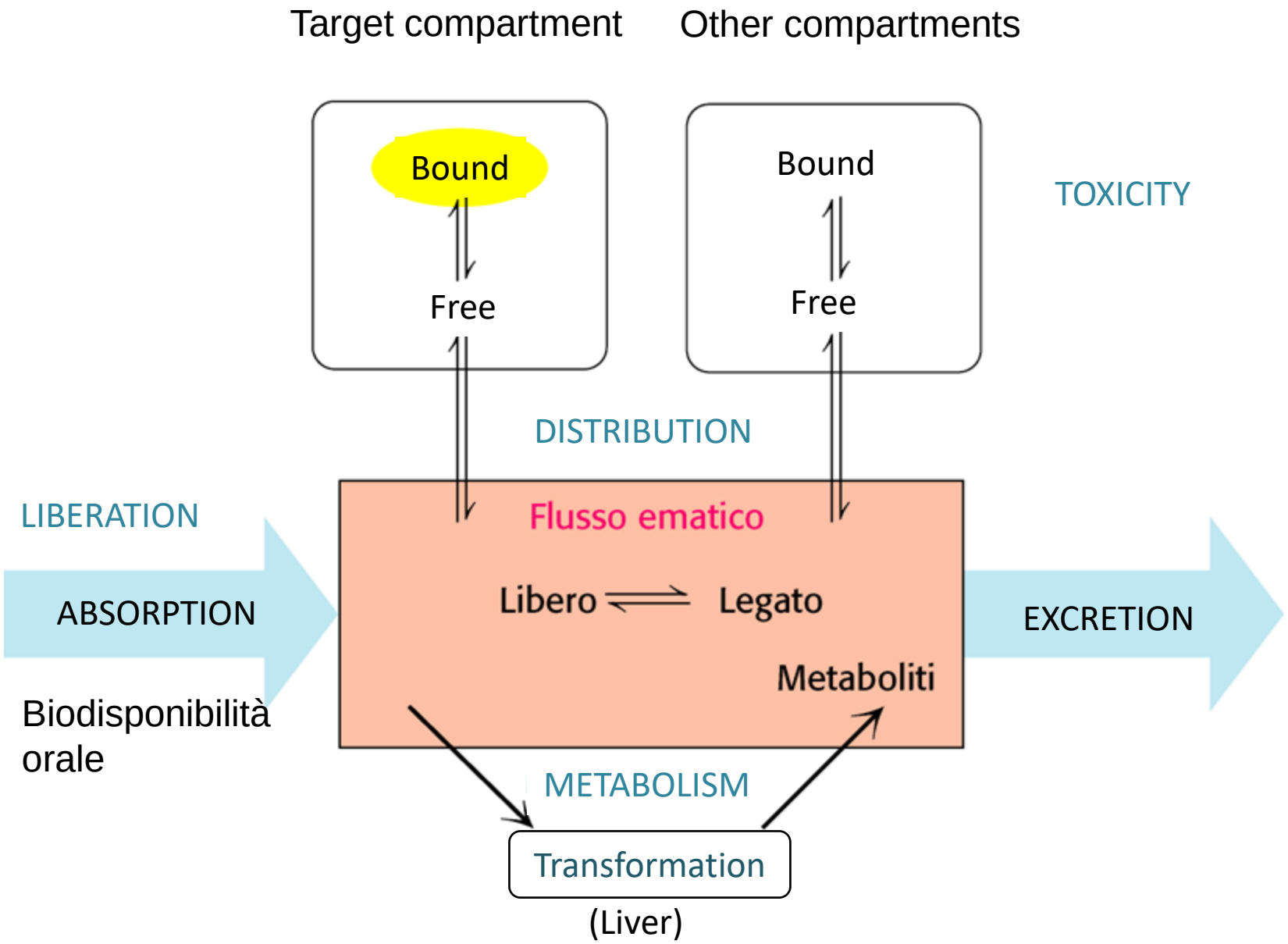
} PHARMACOKINETICS

- TOXICITY – T

PHARMACOTOXICITY

- The ADMET parameters for a molecule depend on its structural and physicochemical characteristics, and how these affect solubility, stability, and interactions with biomolecules and biological systems.

## ADME: Adsorbtion, Distribution, Metabolism, Escretion, Toxicity



## ADME – drug Adsorption and Distribution

- Lipinski's rule of 5 ( LRO5; empirical - formulated at Pfizer in 1997 for oral drugs) is a filter applied to chemical-physical characteristics of a compound, calculated a priori to evaluate the potential for pharmaceutical development

  1) MW < 500 2) H-bond acceptors <10 (2x5) 3) H-bond donors <5 4) ClogP < 5

- The majority of drugs registered in the World Drug Index (WDI) fall within these criteria

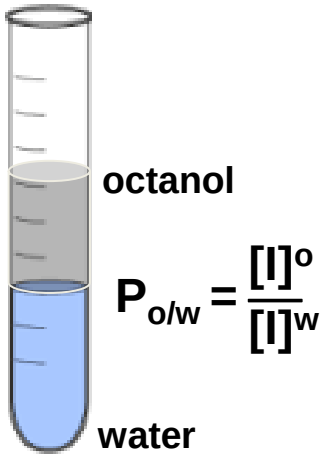
- Violation of at least two of the rules results in a high risk that a drug will not pass development criteria.

log(P) = $\log_{10}$ octanol/water partition coefficient (an index of lipophilicity/hydrophilicity)

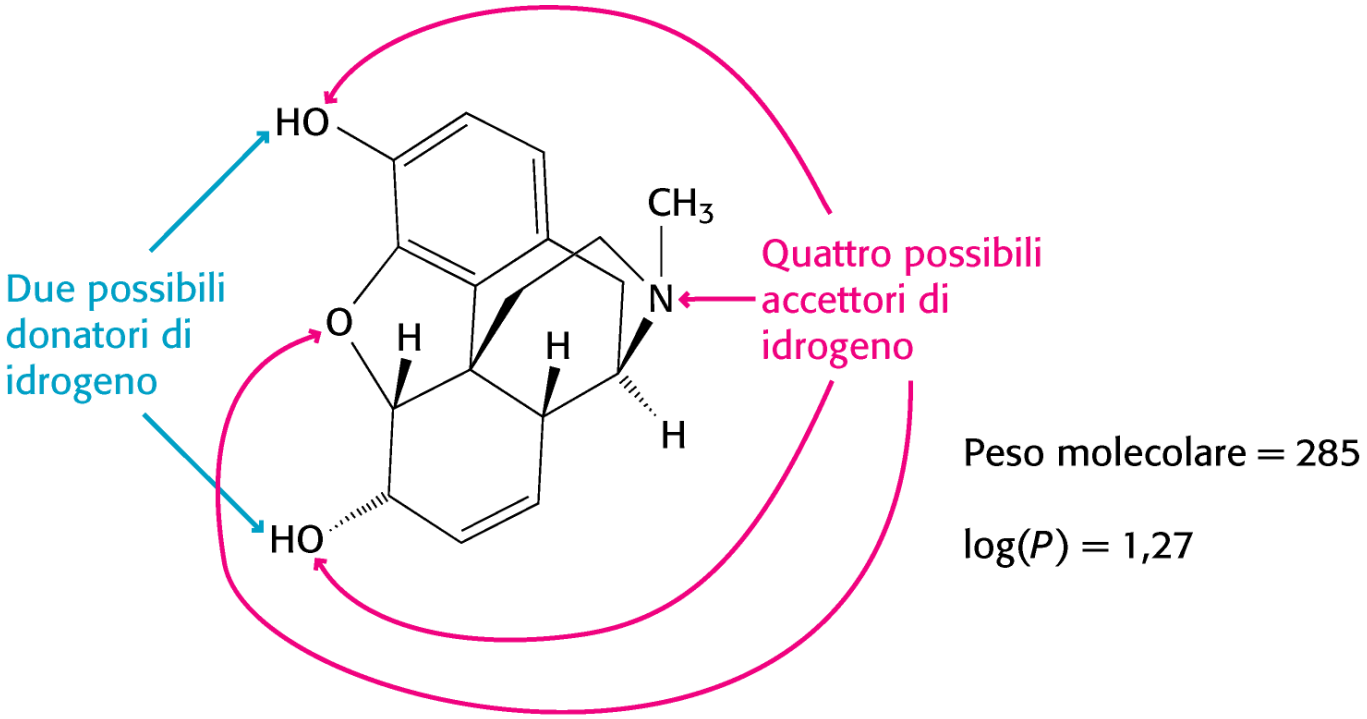
ClogP = logP predicted *in silico* using computational algorithms

*High values of logP allow transmembrane passage but reduce solubility and bioavailability, and the molecules could be sequestered by serum albumin.*

*A compromise is sought (e.g., ideal value for intestinal absorption = 1.4-1.8, for passage through BBB >3).*



- Lipinski's rules applied to morphine




**Oral bioavailability: 33%**

**Bioavailability** = fraction of the drug that enters systemic circulation

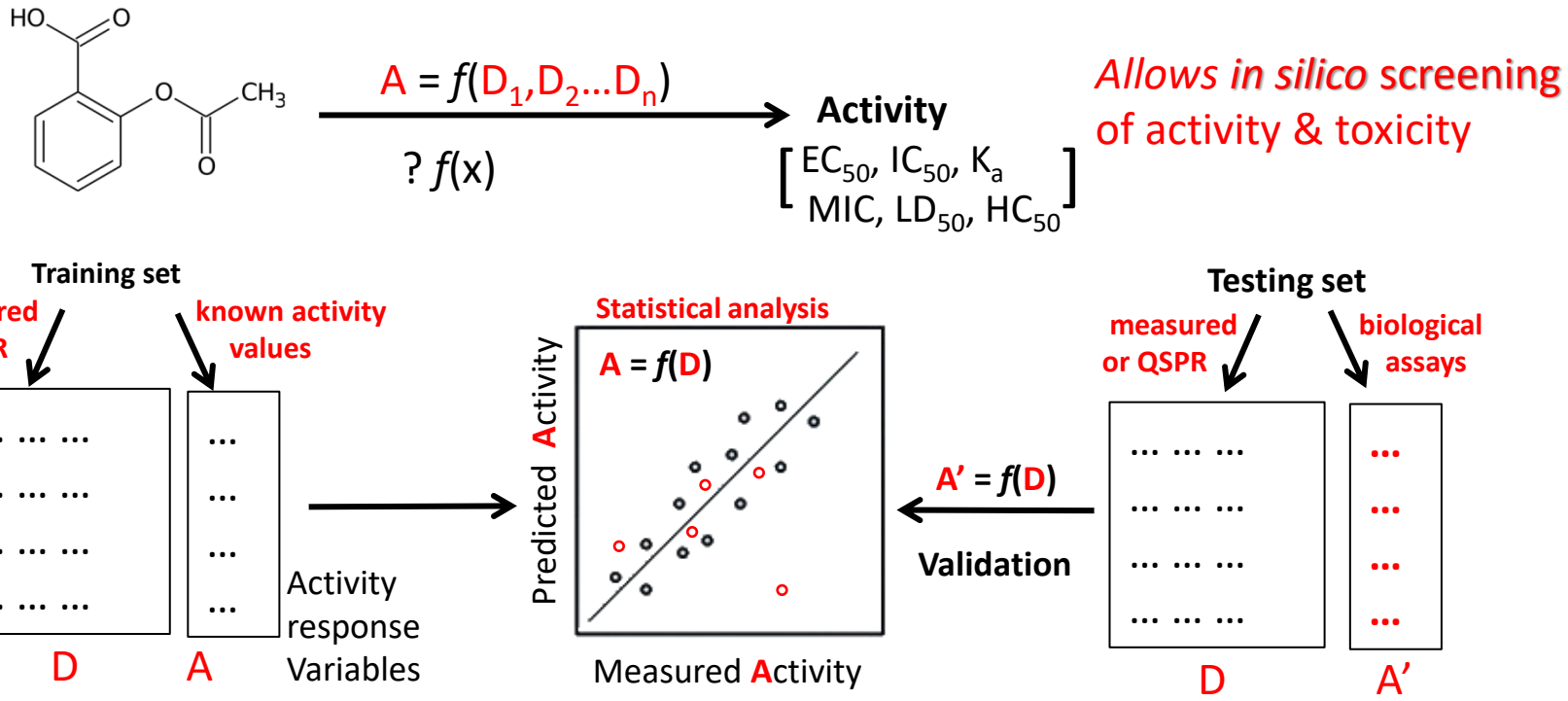
$$\text{Oral bioavailability} = \frac{\text{concentration reaced after oral administration}}{\text{concentration reaced after endovenous administration}}$$

NB. It is important to take into account the fact that morphine metabolism includes alterations/conjugations that alter the parameters, but not necessarily the activity.

# ADMET (cont.)

## In silico methods for estimating ADMET parameters

- Various chemical, physical, and structural descriptors are used in computational models:
- QSPR (Quantitative Structure/Property Relationship) are methods used for precisely estimating physicochemical characteristics (e.g., solubility, lipophilicity), often by summing considering molecule fragments and summing the results
- QSAR (Quantitative Structure/Activity Relationship) are methods for relating structural and physicochemical characteristics (Descriptors) to quantifiable biological/cytotoxic Activities, which allow for prediction of efficacy and toxicity.




$EC_{50}$ = hemimaximal efficacy concentration; $IC_{50}$ = h.m. inhibitory conc.; $K_a$ = binding affinity; $LD_{50}$ = h.m. lethal dose