Natural Products & Drug Discovery

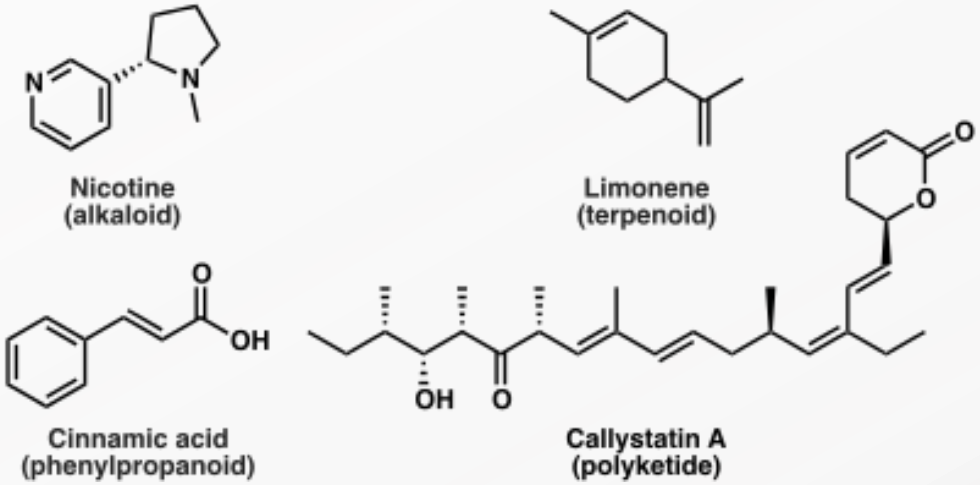
Asst. Prof. Dr. Tanatorn Khotavivattana
Center of Excellence in Natural Products Chemistry
Department of Chemistry, Faculty of Science, Chulalongkorn University
tanatorn.k@chula.ac.th

Group Website

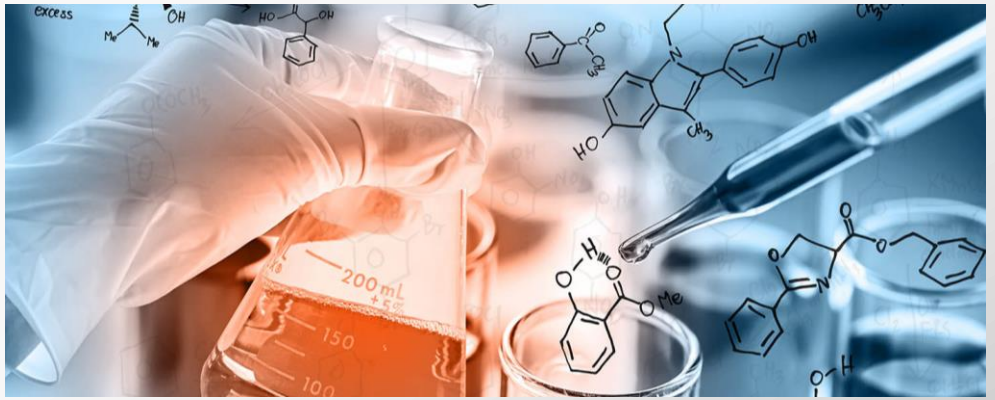
Natural Products

Organic compounds produced by a living organism

Isolation



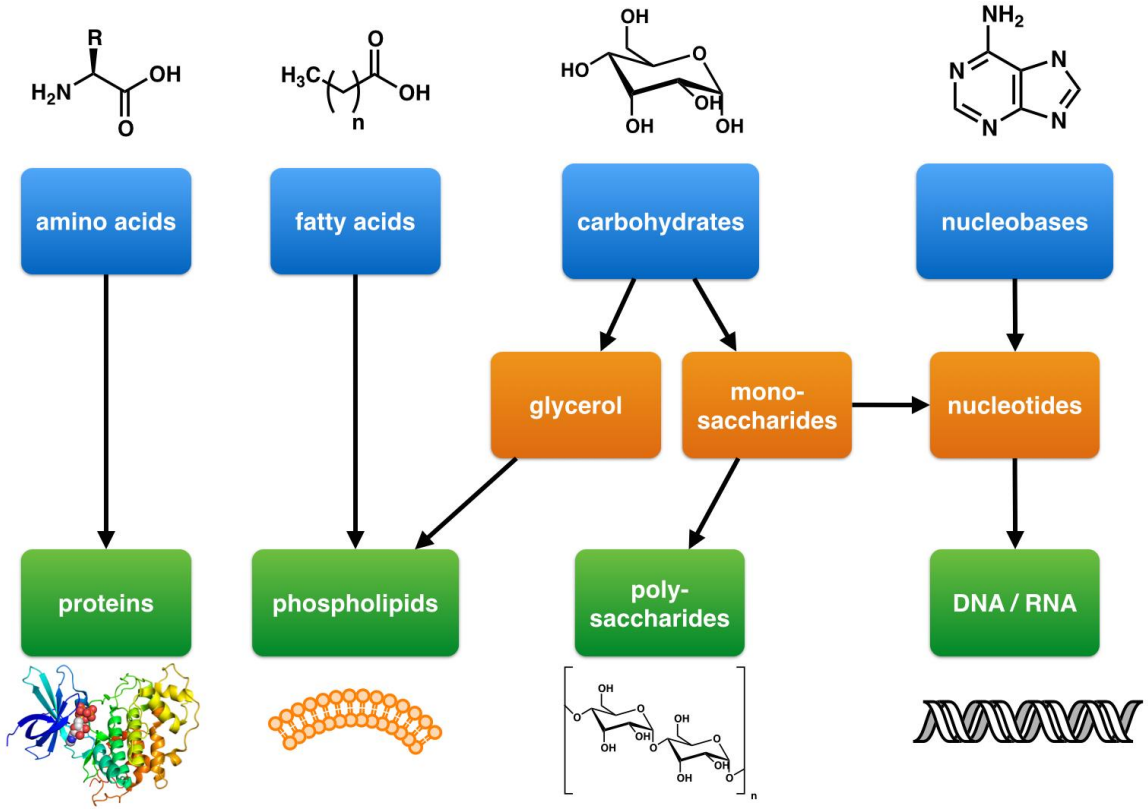
Synthesis



Natural Products - Functions

Primary Metabolites

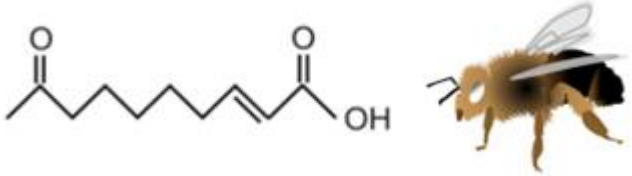
Have an intrinsic function that is essential to the survival of the organism



Secondary Metabolites

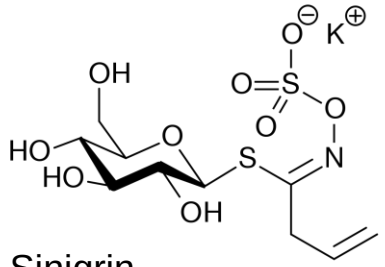
Have an extrinsic function that mainly affects other organisms - Not essential to survival but do increase the competitiveness of the organism within its environment

Pheromones



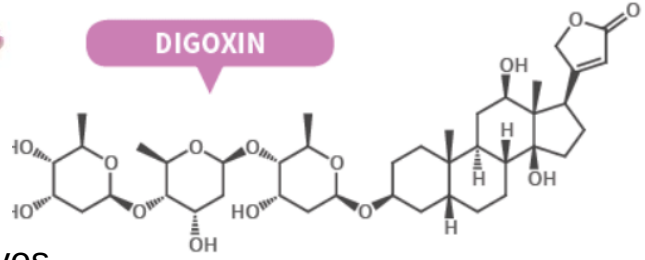
Honeybee (*Apis mellifera*)
(E)-9-oxo-2-decenoic acid

Allelochemicals



Sinigrin
Garlic mustard

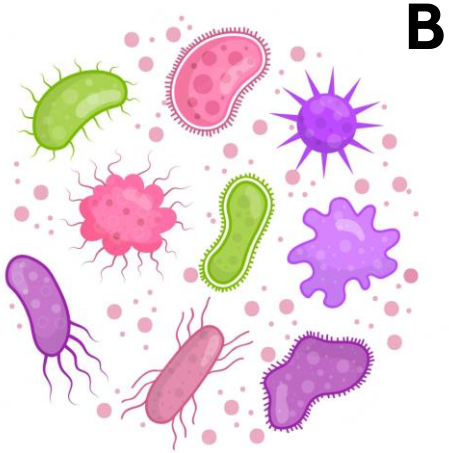
Toxins

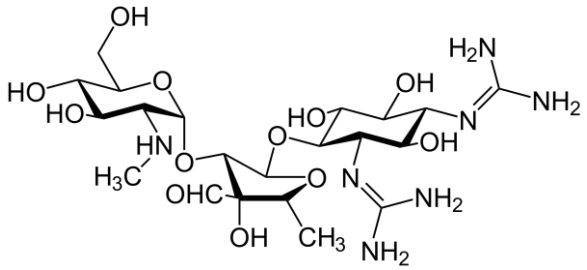


Foxgloves

Natural Products - Sources

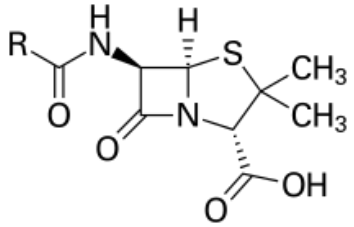
Bacteria





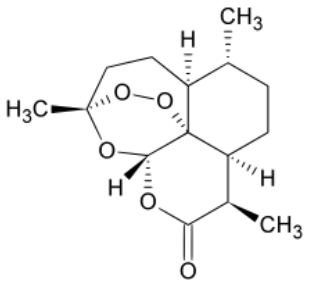
Streptomycin
Antibiotic

Fungi



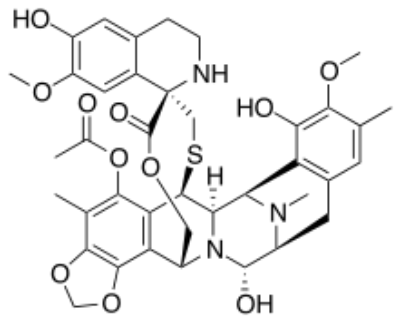
Penicillin
Antibiotic

Plants



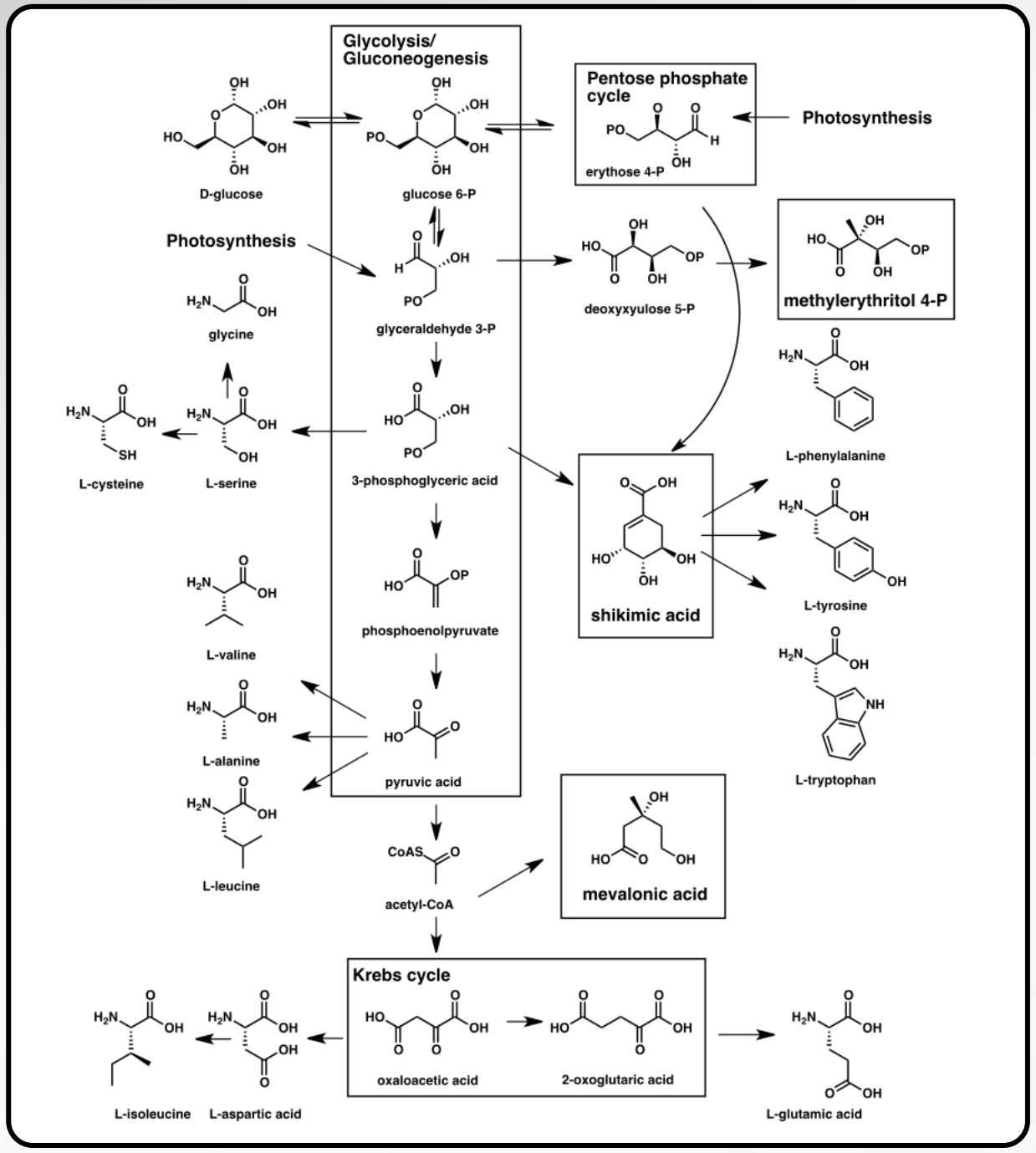
Artemisinin
Antimalarial

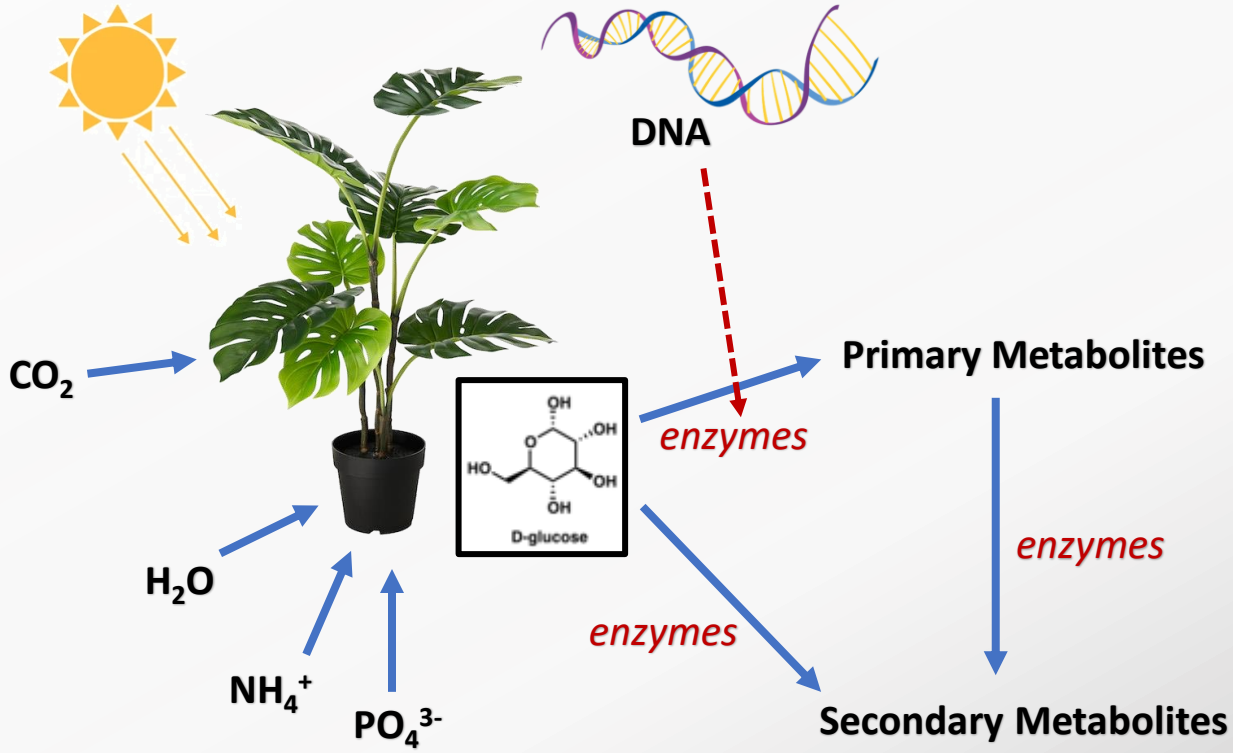
Animals



Trabectedin
Anticancer

Biosynthesis of Natural Products



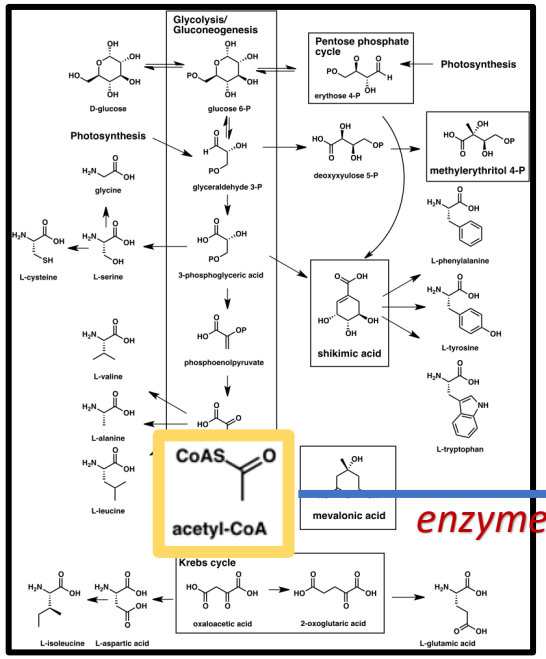


Plant Secondary Metabolites

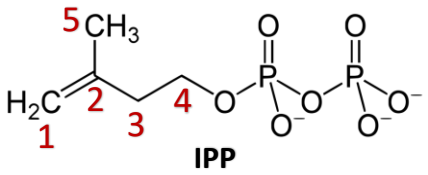
1. Terpenoids

- **isoprene unit(s)**

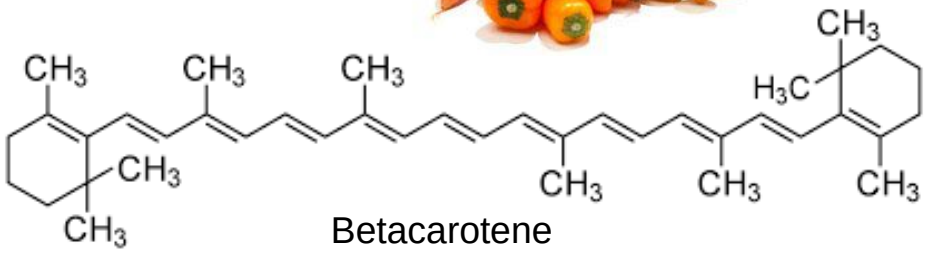
Number of isoprene units	Name	Carbon atoms
1	Hemiterpene	C_5
2	Monoterpene	C_{10}
3	Sesquiterpenes	C_{15}
4	Diterpene	C_{20}
5	Sesterterpene	C_{25}
6	Triterpene	C_{30}
7	Sesquarterterpene	C_{35}
8	Tetraterpene	C_{40}
More than 8	Polyterpene	



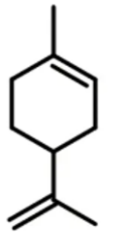
enzymes



enzymes



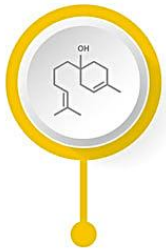
Betacarotene
C_{40}



Limonene
C_{10}

TERPENES IN CANNABIS

THERE ARE MORE THAN 100 TERPENES IN JUST ONE CANNABIS FLOWER. HERE ARE SOME OF THE MOST WELL KNOWN TERPENES RIGHT NOW, MOST OF WHICH YOU'LL FIND IN LEGAL CANNABIS PRODUCTS IN YOUR AREA.

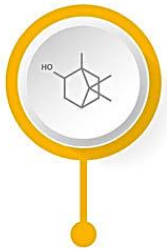


Bisabolol

floral

Properties
anti-inflammatory
anti-irritant
anti-microbial

Common Uses
cancer, skin lesion

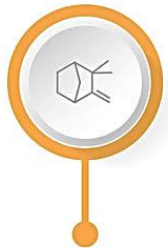


Borneol

mint

Properties
anti-inflammatory
antinociceptive

Common Uses
eyesight, pain relief



Camphene

fir needles, musky earth

Properties
anti-oxidant
skin lesion

Common Uses
cardiovascular diseases

Caryophyllene

spicy

Properties
anti-bacterial
anti-inflammatory
anti-fungal

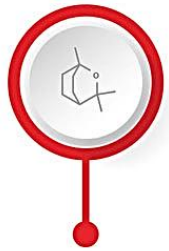
Common Uses
insomnia, muscle spasms
pain relief

Delta 3 Carene

pine, rosemary

Properties
anti-inflammatory
bone stimulant

Common Uses
memory

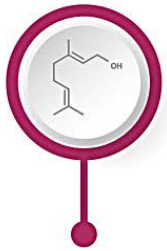


Eucalyptol

mint

Properties
anti-bacterial
anti-fungal

Common Uses
alzheimer's
pain Relief



Geraniol

peach, rose grass

Properties
anti-cancer
anti-oxidant
neuroprotectant

Common Uses
cancer, pain relief

Humulene

earthy

Properties
anti-bacterial
anti-inflammatory
anti-tumor effects

Common Uses
cancer, infections
appetite suppression

Limonene

bitter citrus

Properties
anti-anxiety
anti-cancer
digestion, gallstones

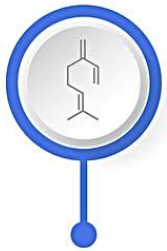
Common Uses
liver detoxification
weight loss, sleep aid

Linalool

floral

Properties
anti-anxiety
anti-epileptic
anti-psychotic
pain killing

Common Uses
depression, convulsions
insomnia, pain relief



Myrcene

citrus, cloves

Properties
relaxing
sedating

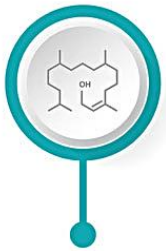
Common Uses
inflammation, insomnia
spasms, pain

Pinene

pine

Properties
anti-depressant
anti-inflammatory
anti-microbial

Common Uses
asthma, bronchitis
cancer, depression
memory, mental alertness



Phytol

balsamic, floral

Properties
anti-insomnia
immunosuppressant

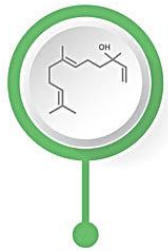
Common Uses
reduce itching
sleep aid
wound healing

Terpinolene

smoky, woody

Properties
anti-bacterial
anti-fungal
anti-insomnia
antiseptic

Common Uses
cancer
heart disease
sleep aid

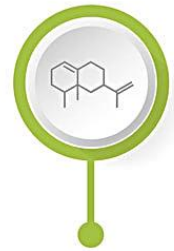


Trans-nerolidol

citrus, rose

Properties
anti-cancer
anti-microbial
anti-oxidant,
anti-parasitic

Common Uses
relaxing
skin lesion



Valencene

sweet citrus

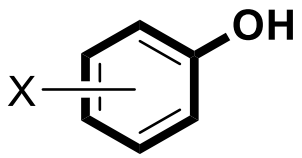
Properties
anti-inflammatory
anti-melanogenesis
antiallergic

Common Uses
memory
skin lesion

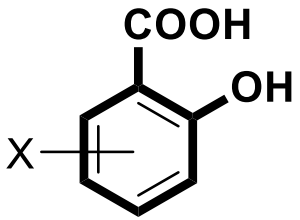
Plant Secondary Metabolites

2. Phenolic

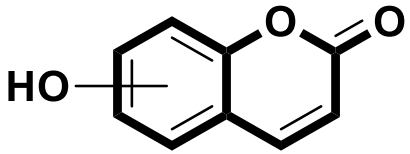
- **Contain phenol ring(s)**



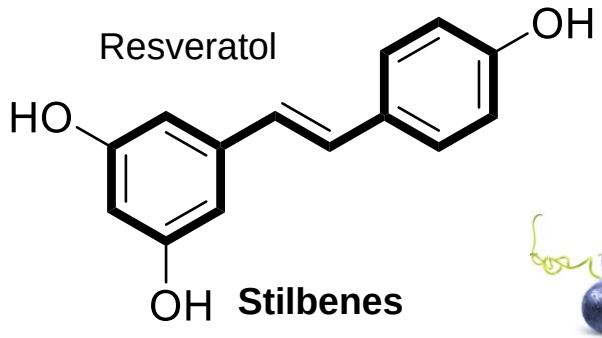
Phenols



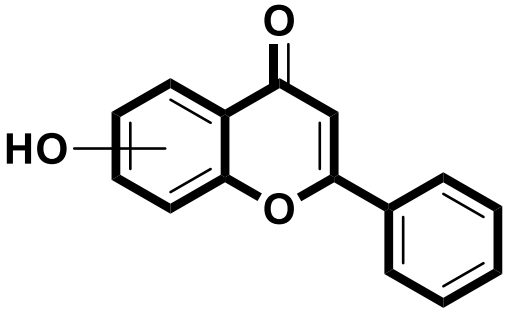
Phenolic acids



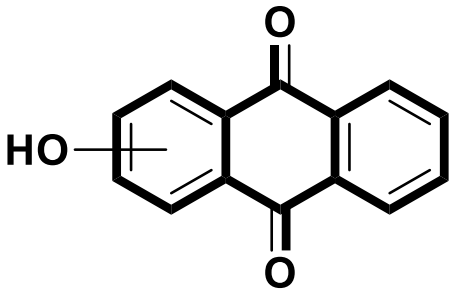
Coumarins



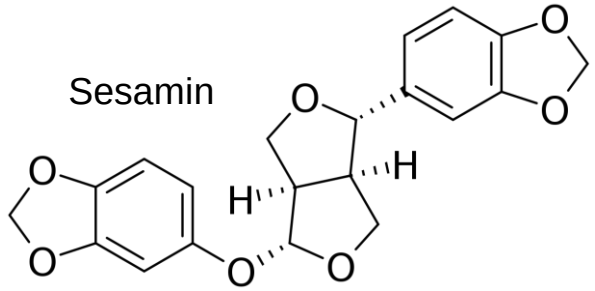
Stilbenes



Flavonoids



Xanthones

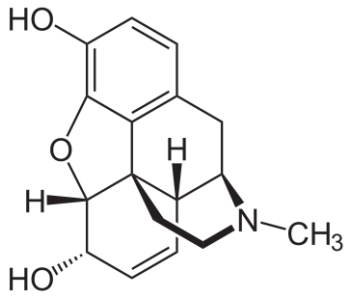


Lignans

Plant Secondary Metabolites

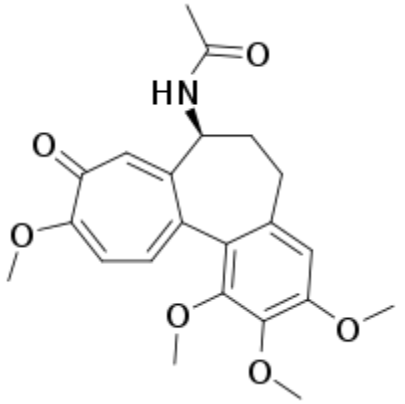
3. Alkaloids

- **Contain nitrogen atom(s)**



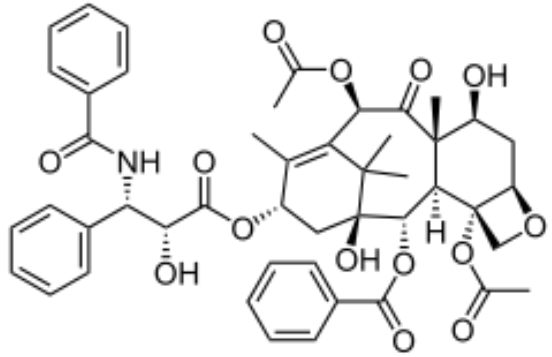
Morphine

Pain medication



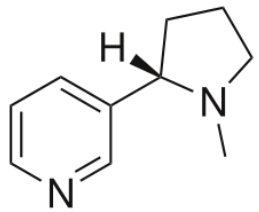
Colchicine

Treat gout and Behçet's disease



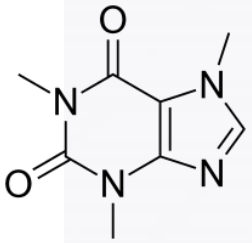
Paclitaxel (Taxol)

Chemotherapy medication



Nicotine

Stimulant and anxiolytic.



Caffeine

Central nervous system (CNS) stimulant

Natural Sources to Crude Extracts

Season

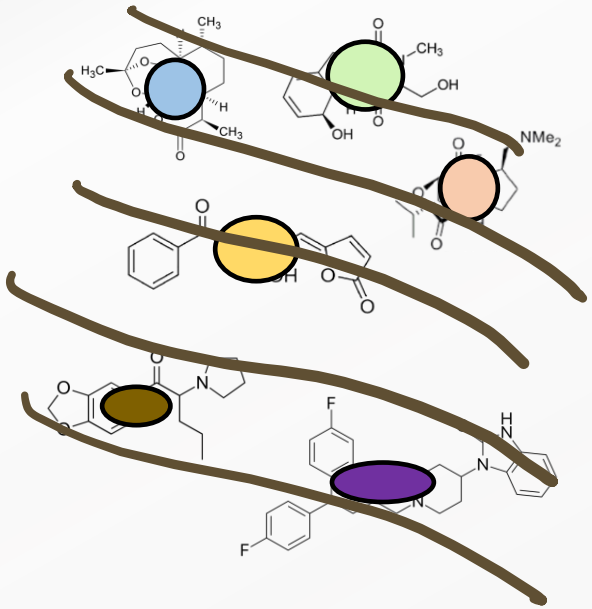
Age

Growing Conditions

Natural Sources

Solvent Extraction

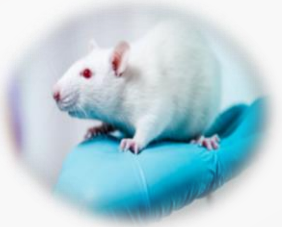
Crude Extracts



Solvent 1

Solvent 2

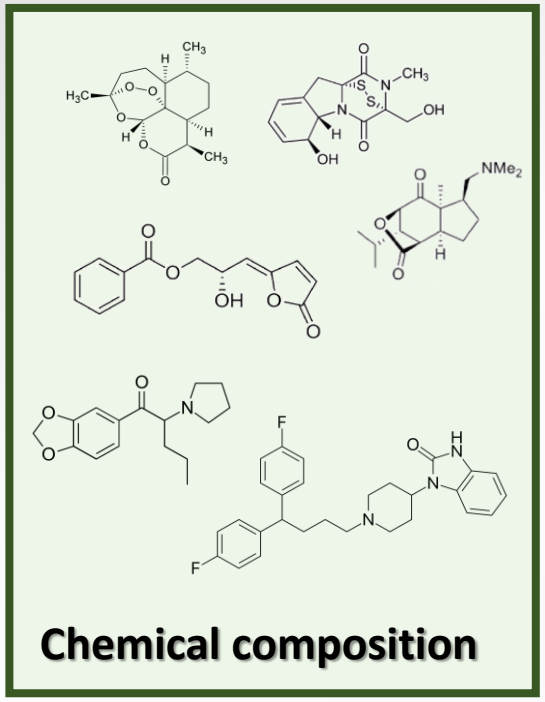
Time / Temperature



Biological Testing

Products

Crude Extracts to Natural Products



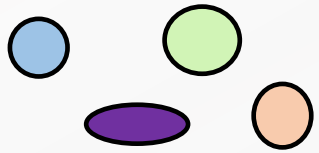
Chemical composition

HPLC-MS

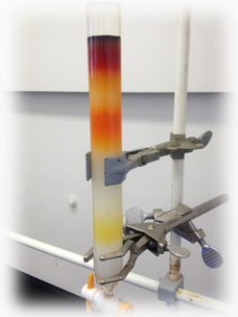
Analysis

GC-MS

Crude Extracts

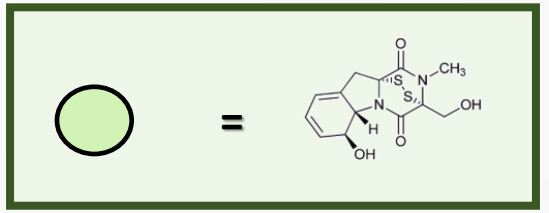


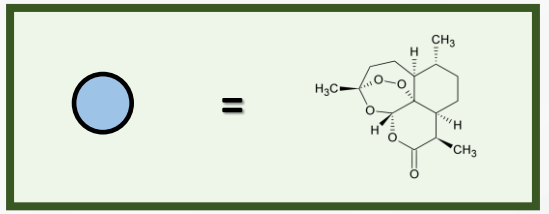
Isolation

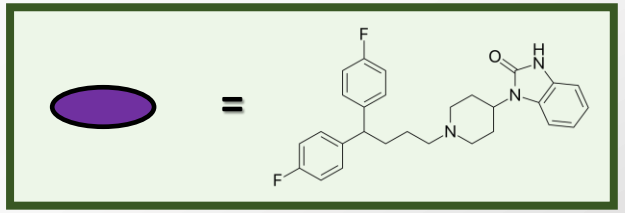


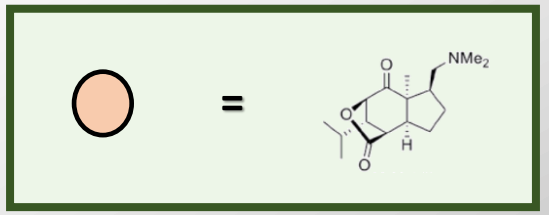
Characterization

NMR, HR-MS

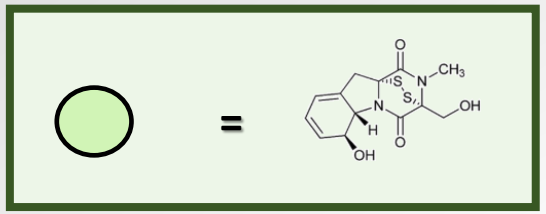


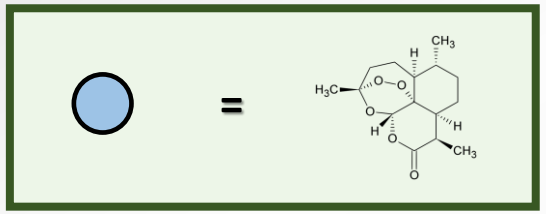


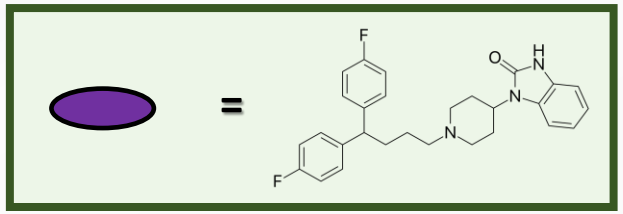


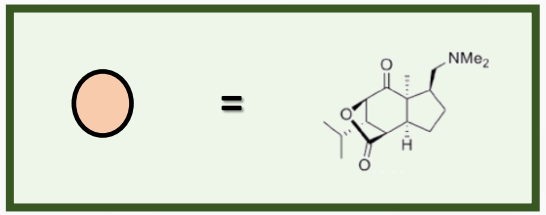


Natural Products to Drugs

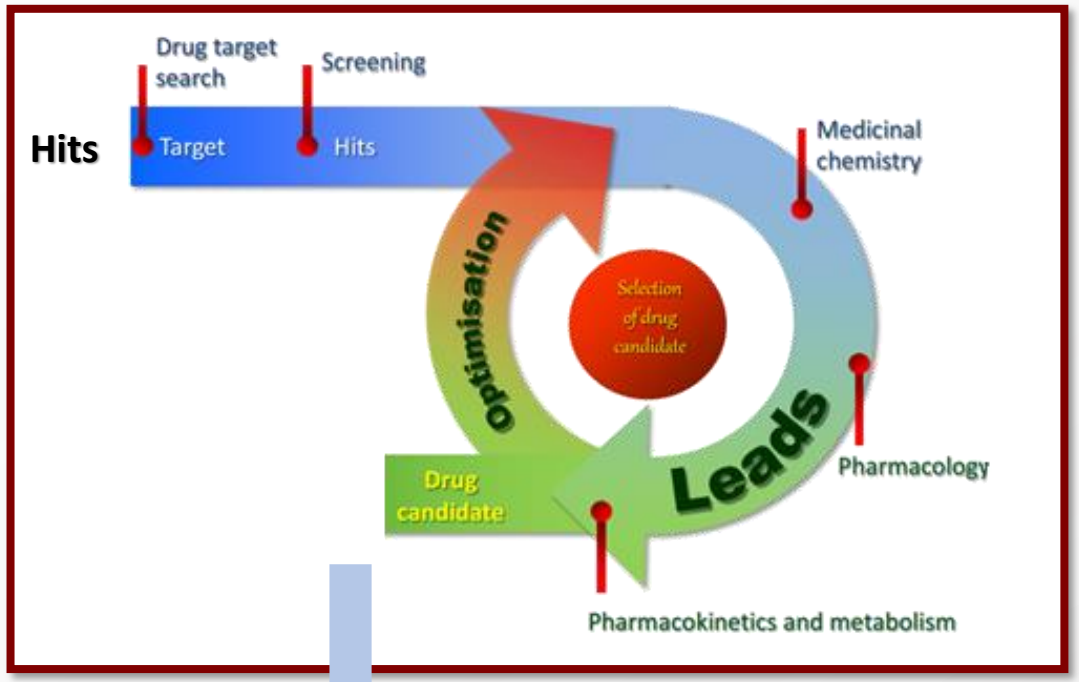


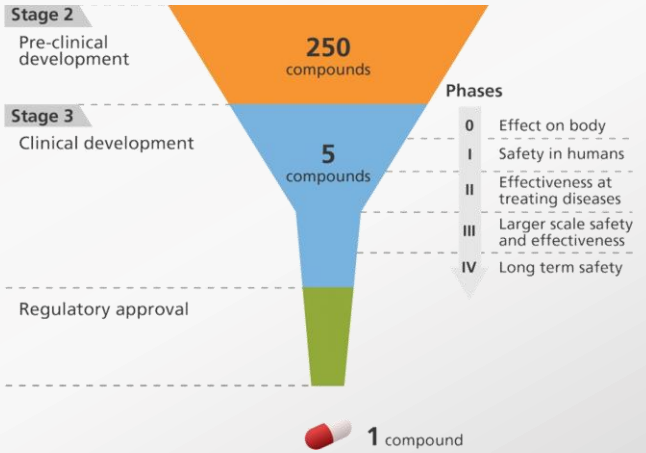






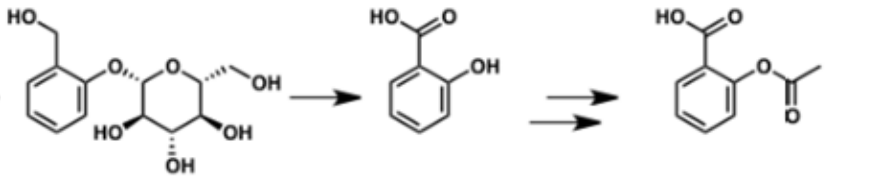
Biological Testing





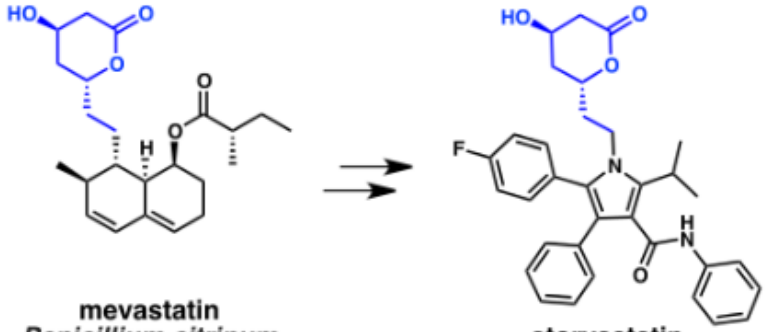
Success Examples

willow bark



salicin → salicylic acid → acetylsalylic acid (aspirin)

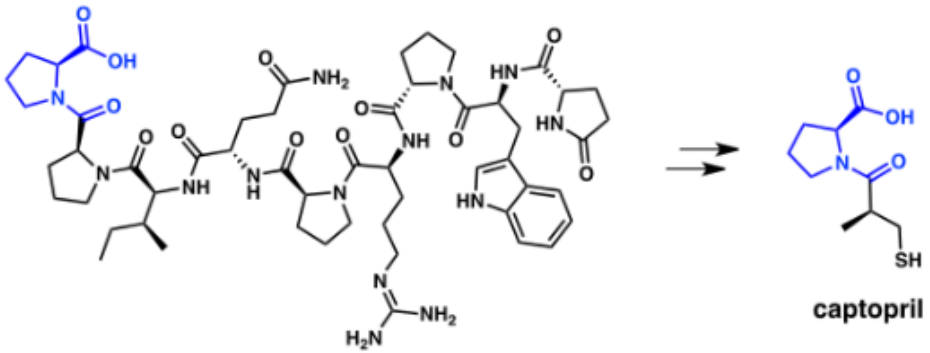
pain relief



mevastatin
Penicillium citrinum

→ atorvastatin

lower cholesterol

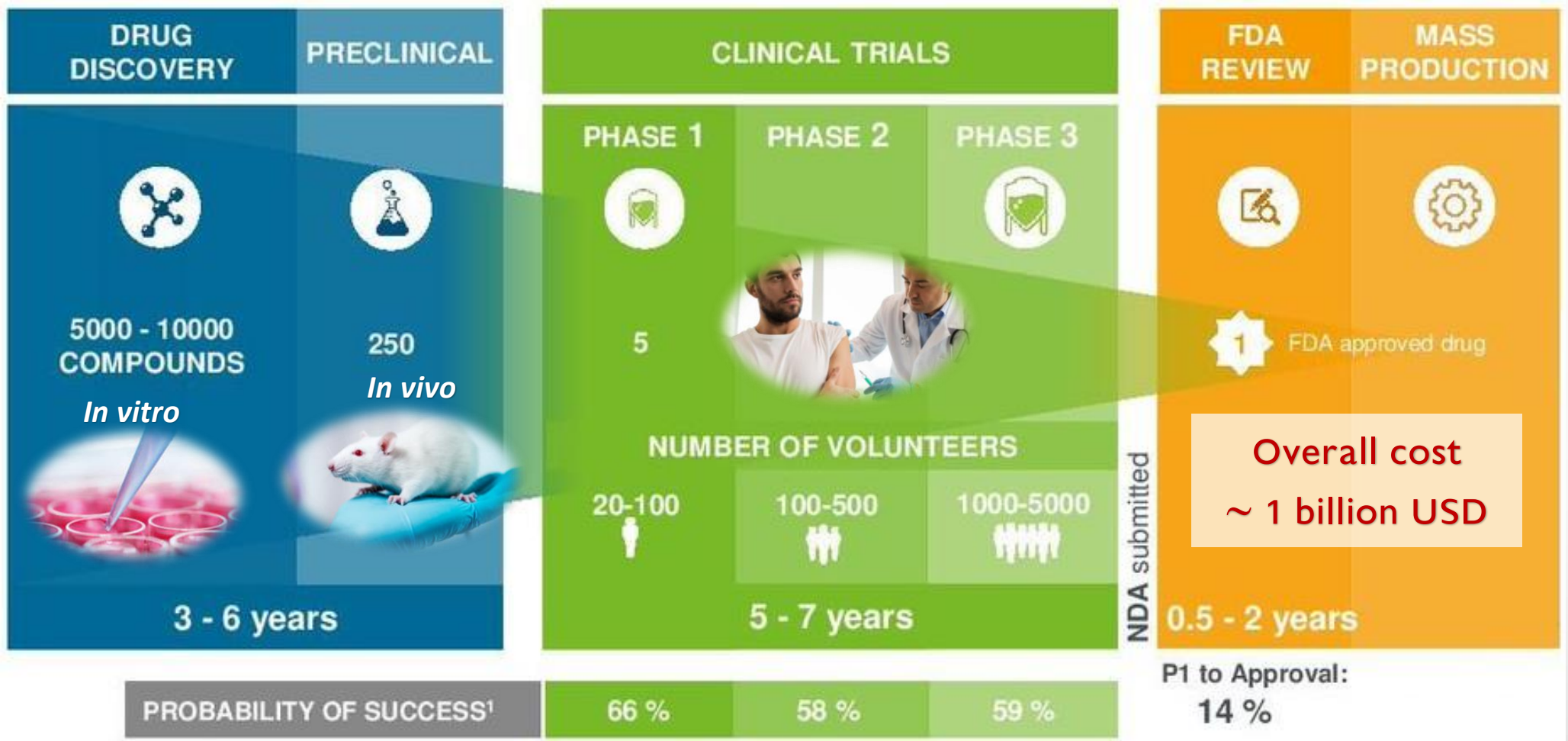


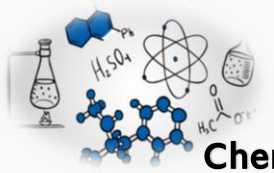
bradykinin potentiating factor (teprotide)
bothrops jararca

captopril

treat hypertension and congestive heart failure

Pharmaceutical Drug Development Process




Chemist

Biologist

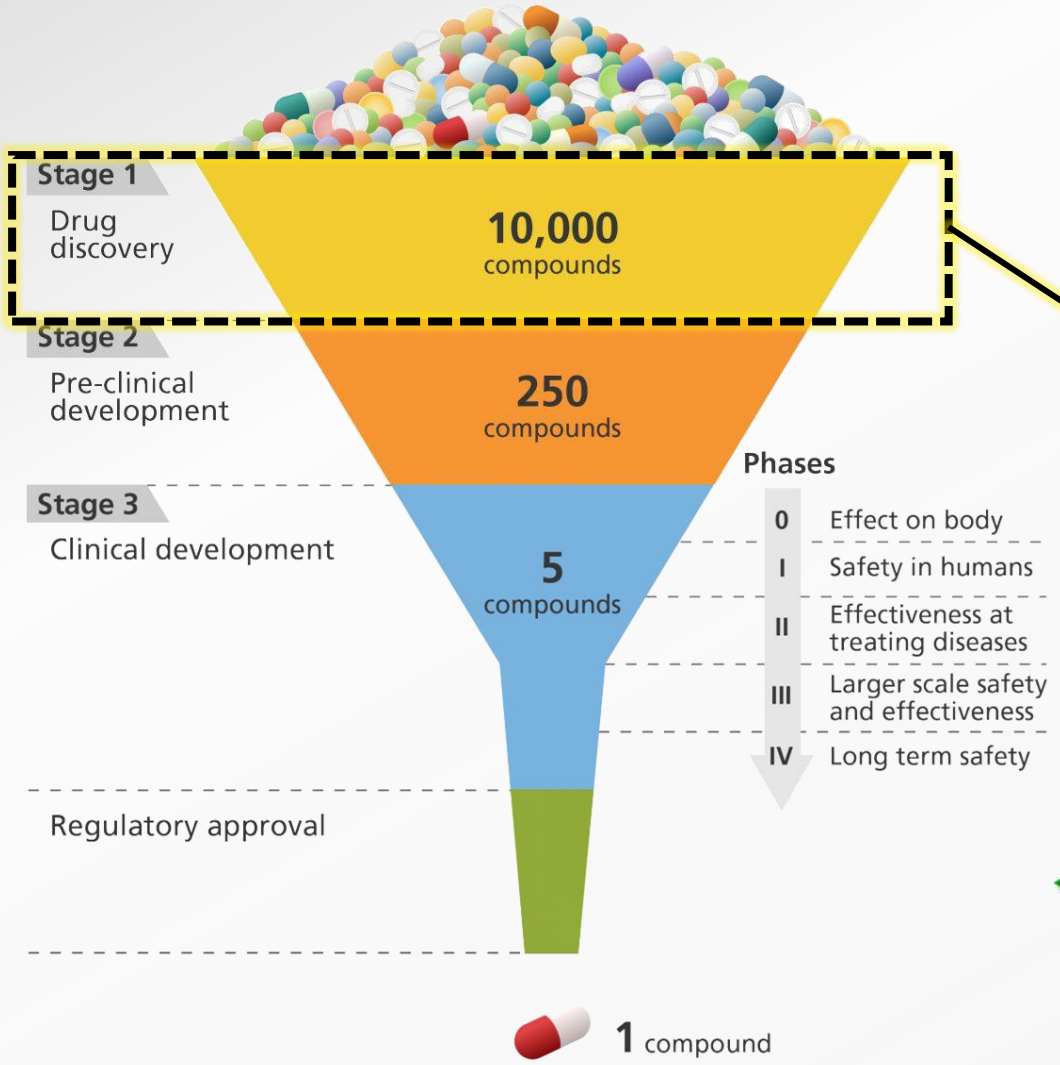


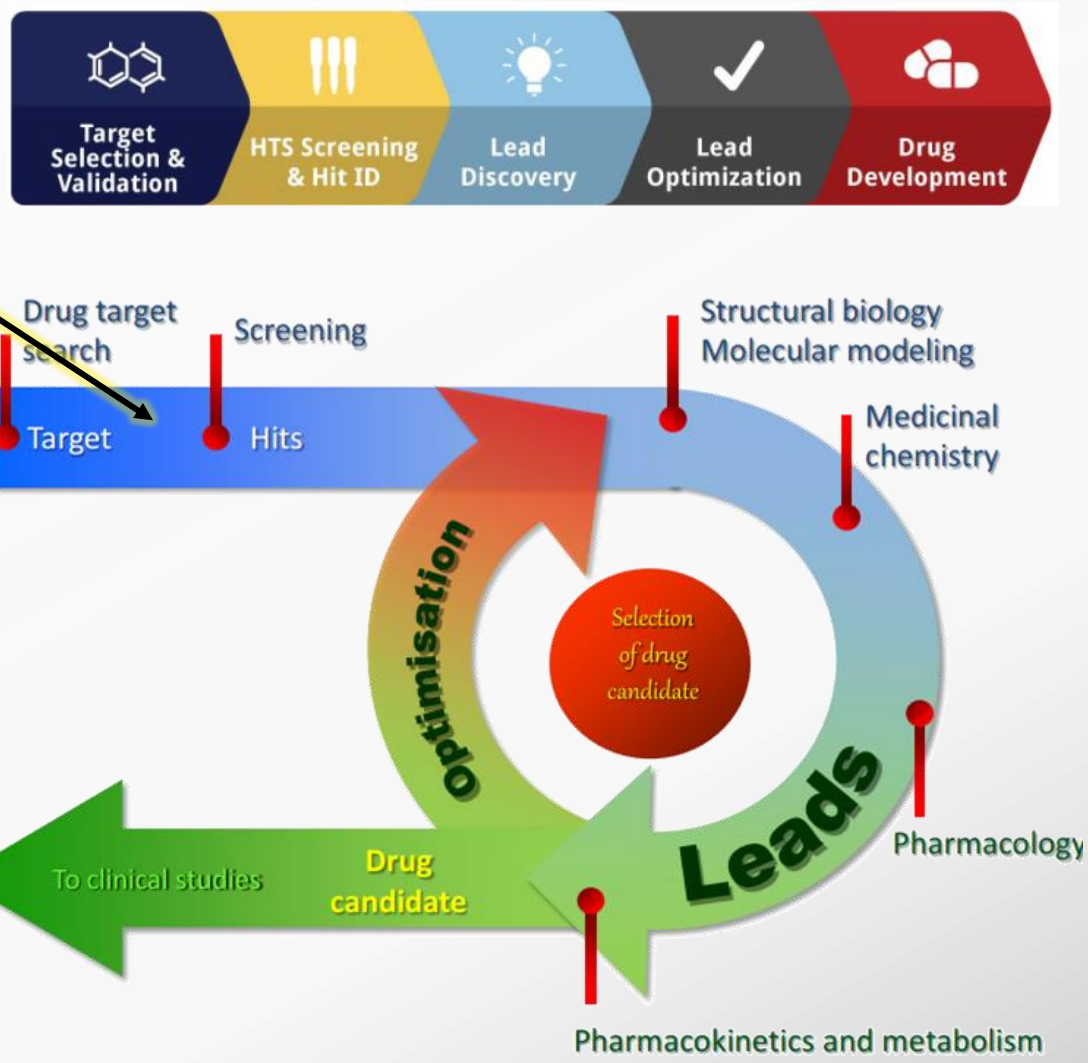
Biochemist

Pharmacologist

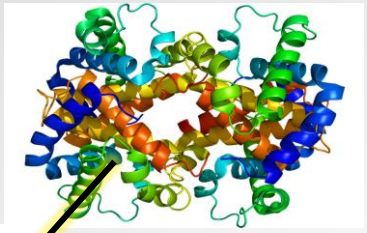

Physician


Drug Discovery and Development Pipeline



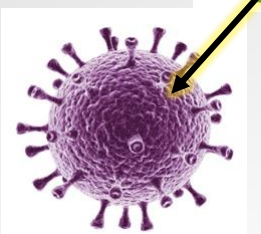


Target Identification and Validation



- **Target-based**

(mostly enzymes)

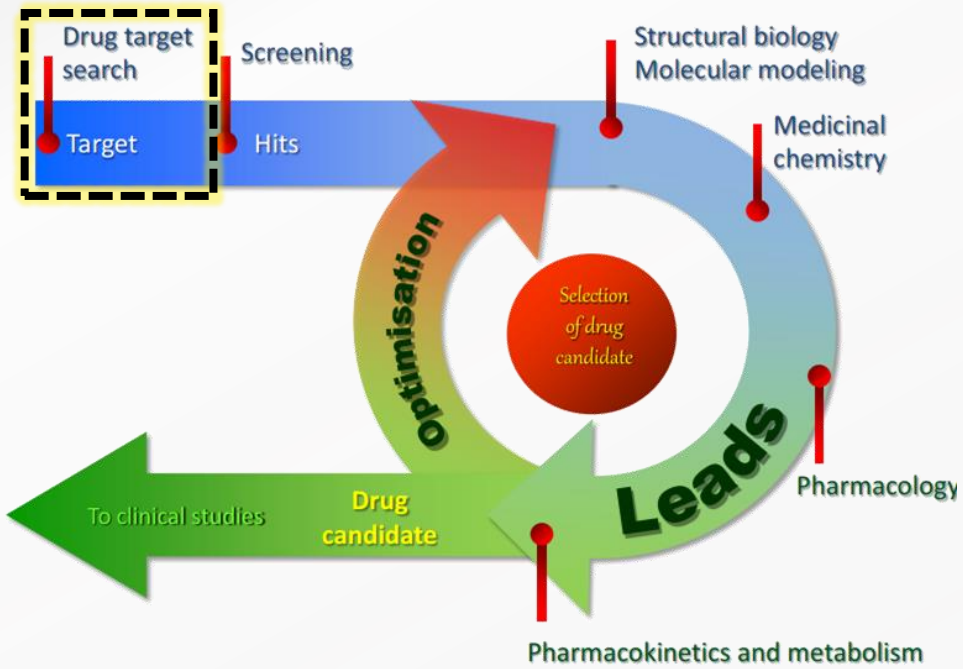


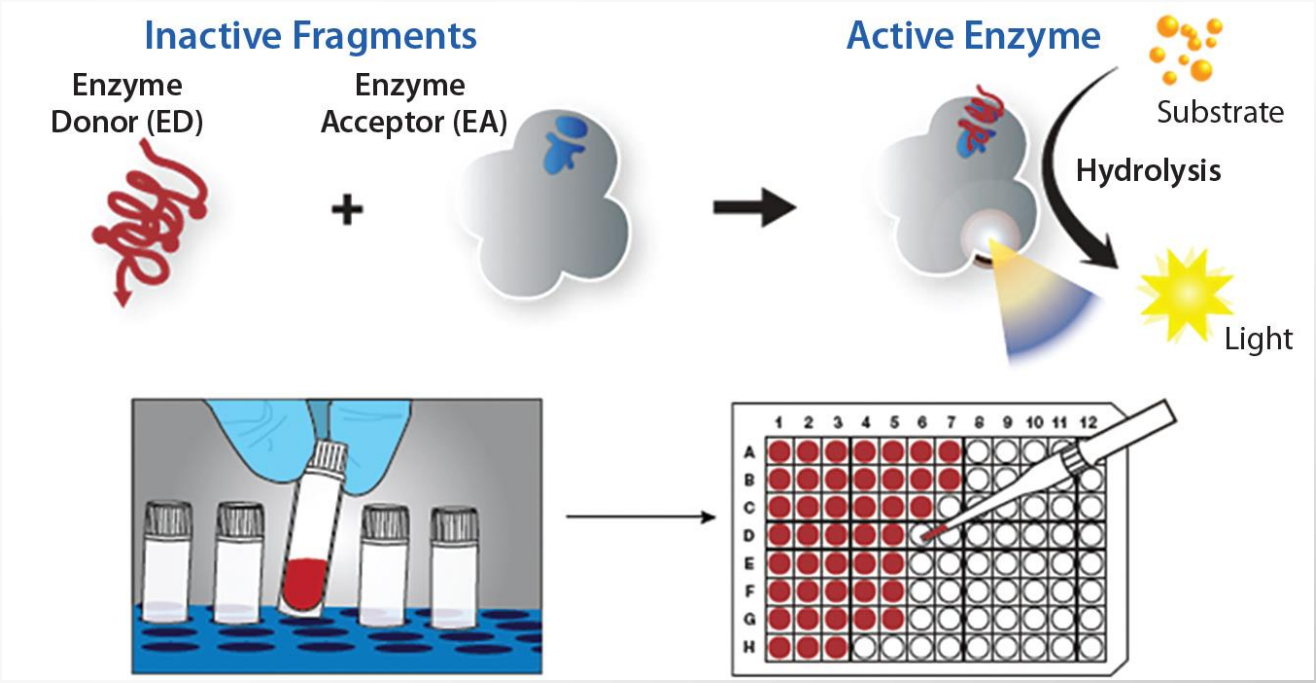
- **Cell-based**

(phenotypic screening)

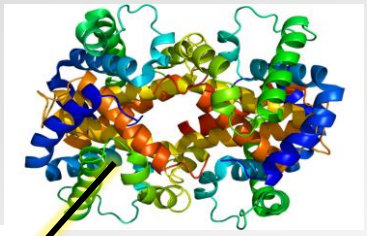
Identifying biological molecular structures and conducting validation experiments to show therapeutic effect

- **TARGET IDENTIFICATION**
- **TARGET VALIDATION**
- **ASSAY VALIDATION**



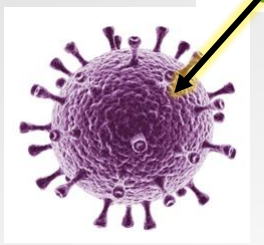


Hit Identification



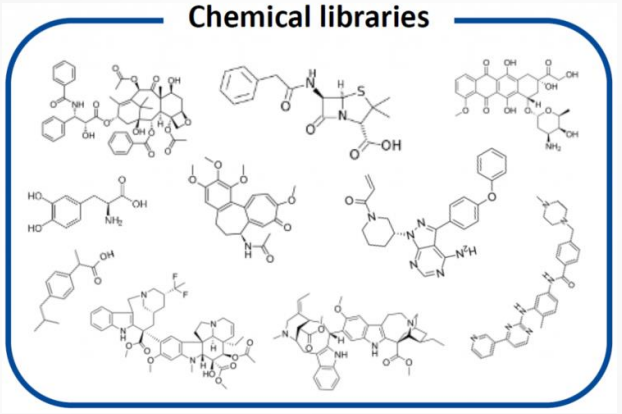
- **Target-based**

(mostly enzymes)

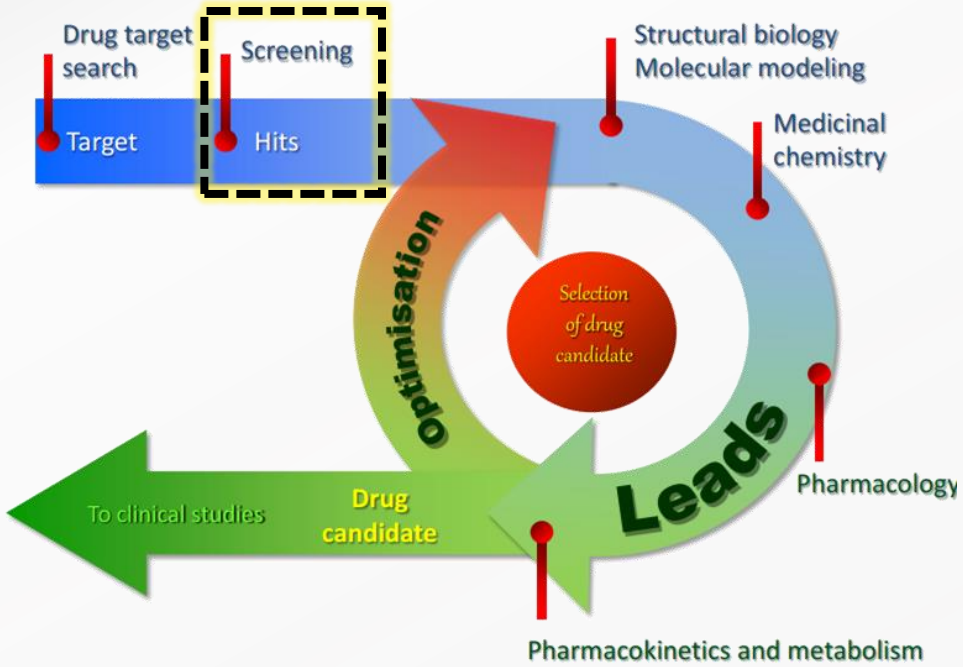


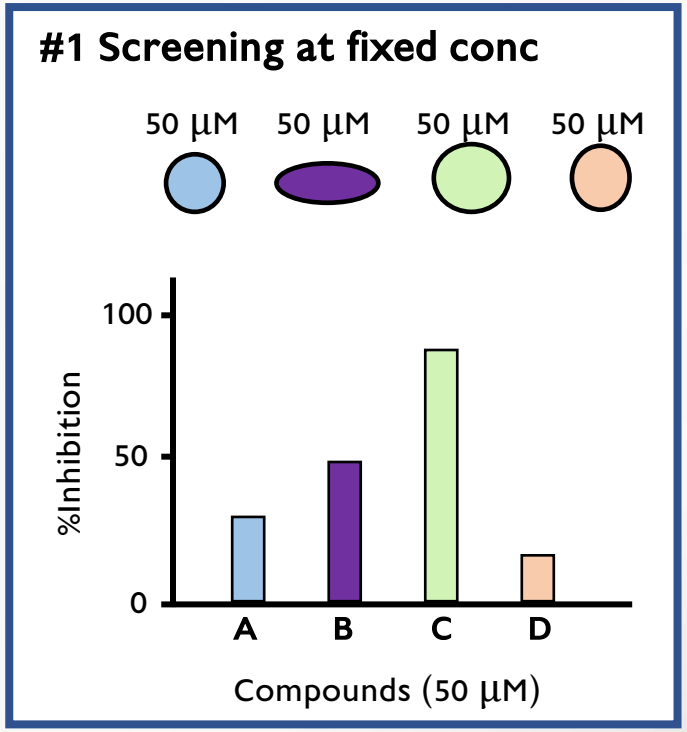
- **Cell-based**

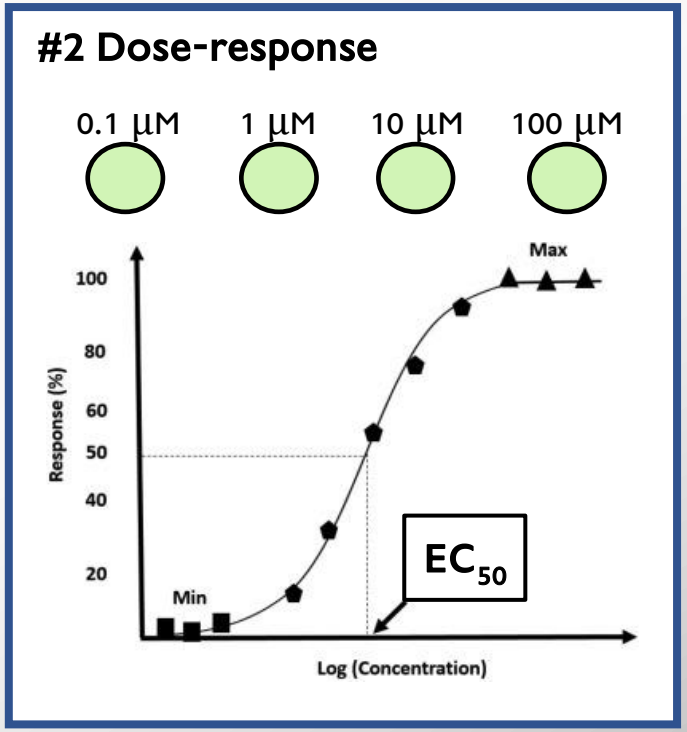
(phenotypic screening)



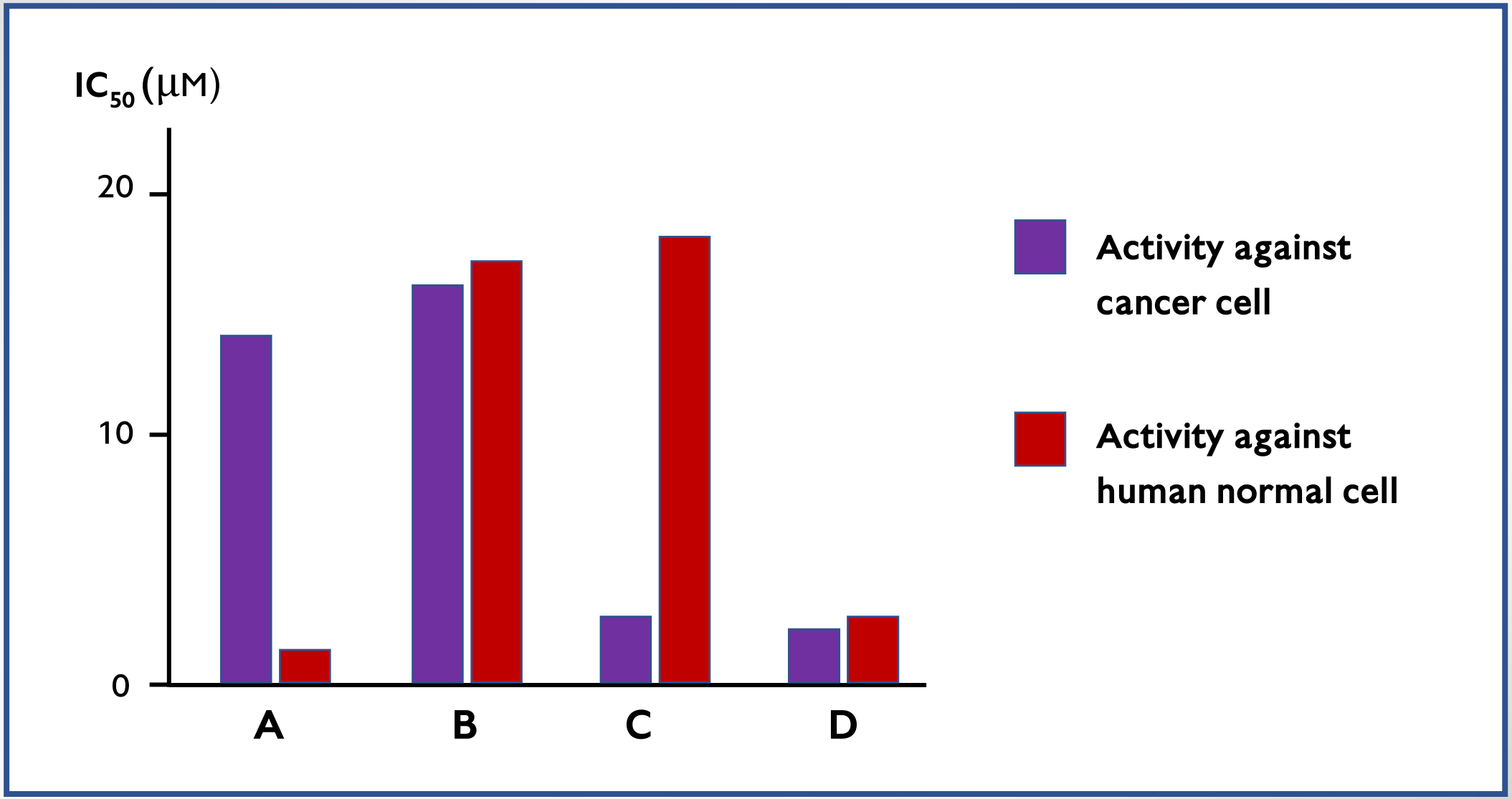
- **High throughput screening**
- **Fragment-based screening**
- **Virtual Screening**
- **Hit Confirmation**







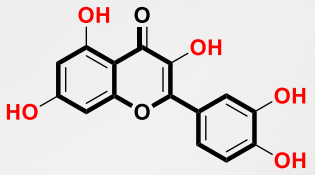
IC_{50}


IC50 (μM)
20
10
0
A
B
C
D
Activity against cancer cell
Activity against human normal cell

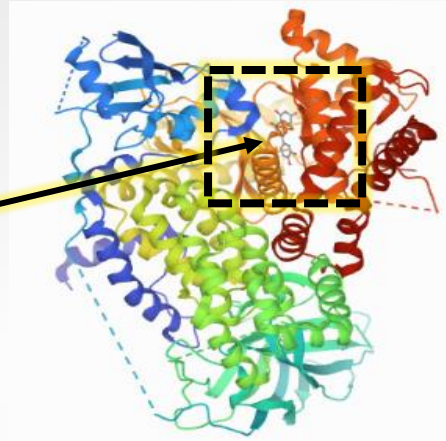
Hit to Lead & Lead Optimization

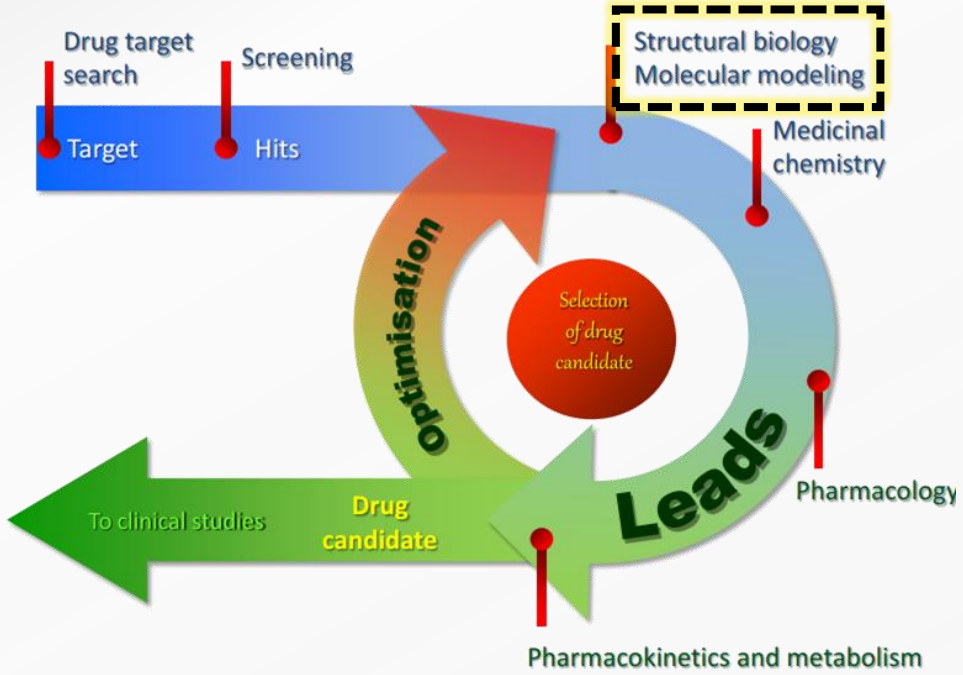
Anti-cancer effect

Hit



Quercetin
(IC_{50} = 12 µM)



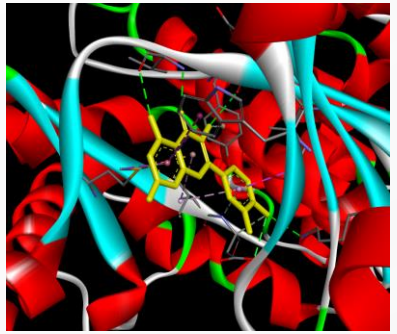


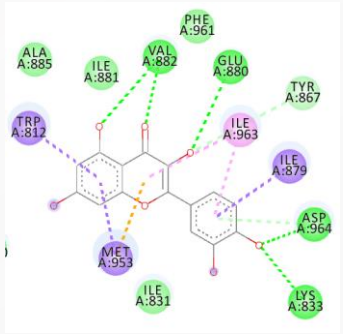
PI3K

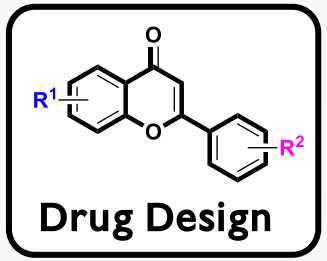
Cell apoptosis, proliferation, cell motility, and adhesion

PI3K inhibition (IC_{50} = 3.8 µM)

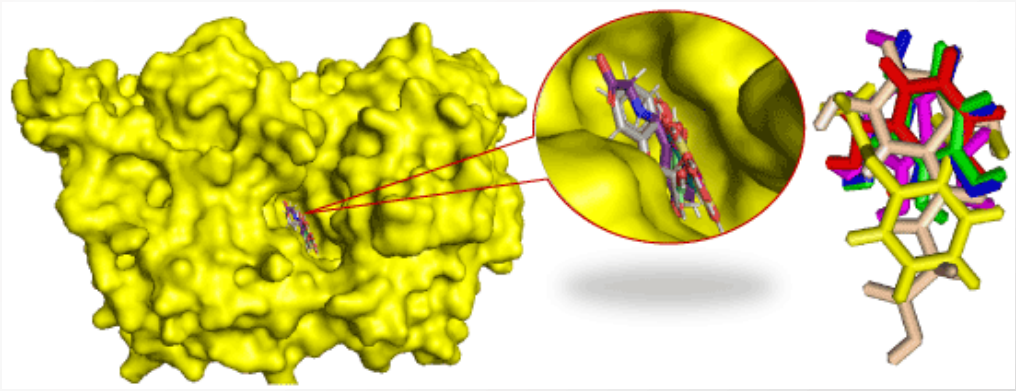
- **Structural Biology – X-Ray structure of co-crystalized complex**



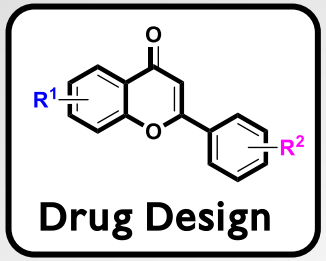




- **Molecular Modeling – Computational Chemistry**

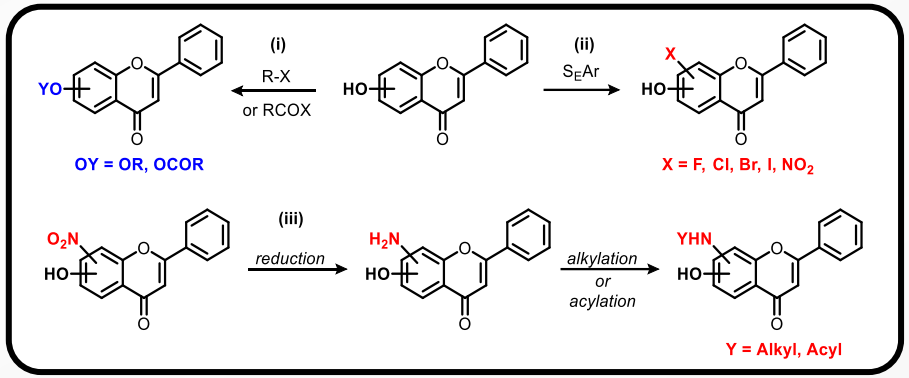


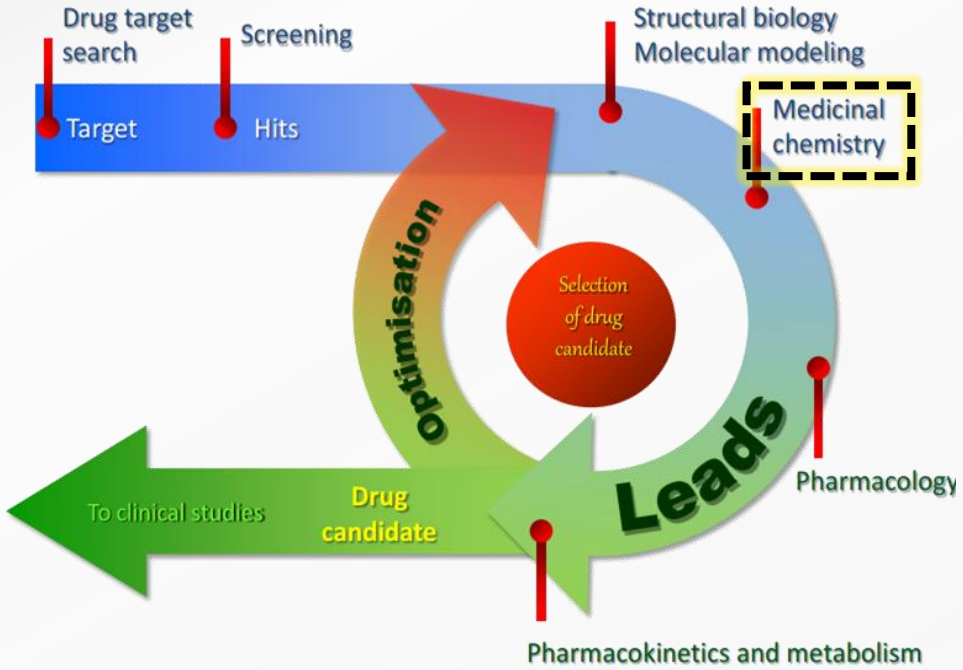
Hit to Lead & Lead Optimization



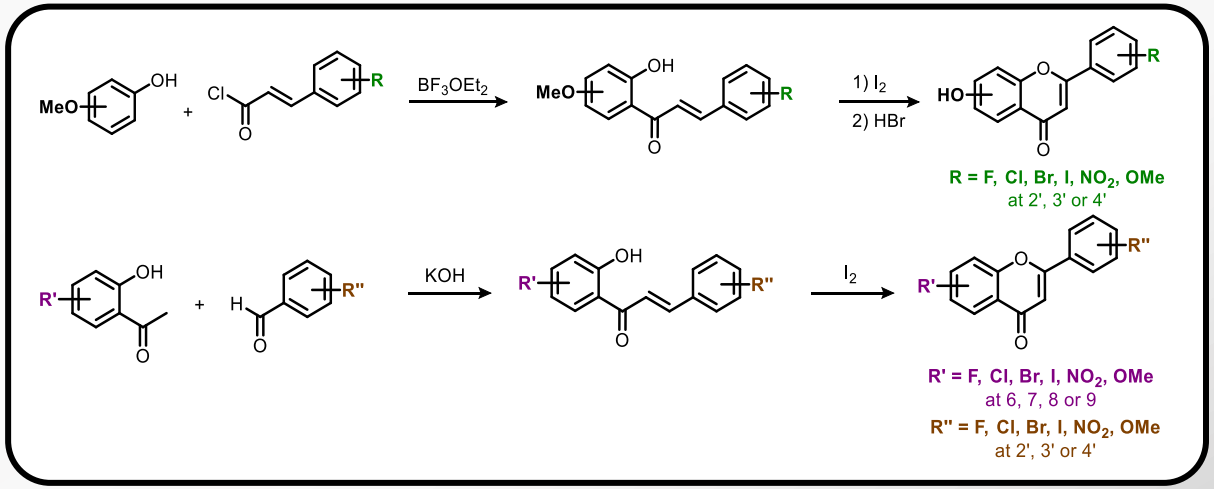
Organic Synthesis

Semi-synthetic Approach



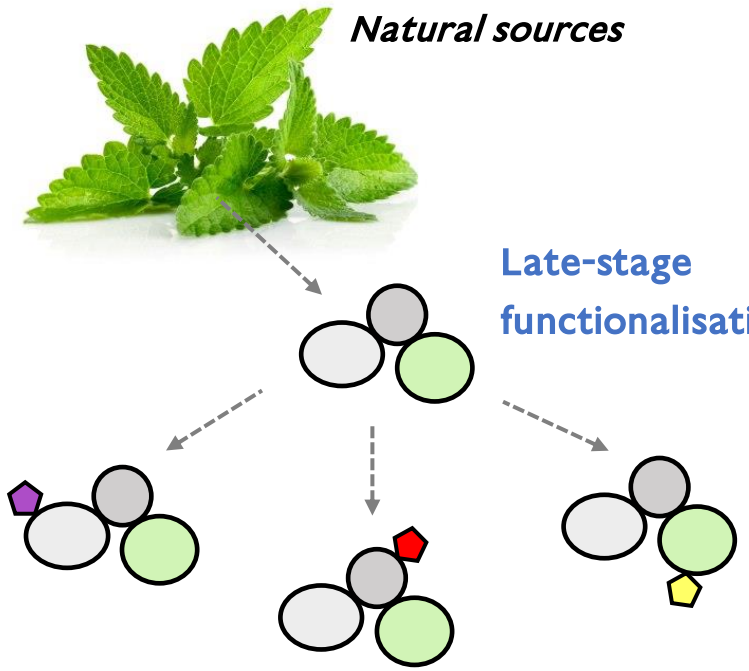


Total Synthetic Approach



Synthesis of Drug Analogs

Semi-Synthesis Approach

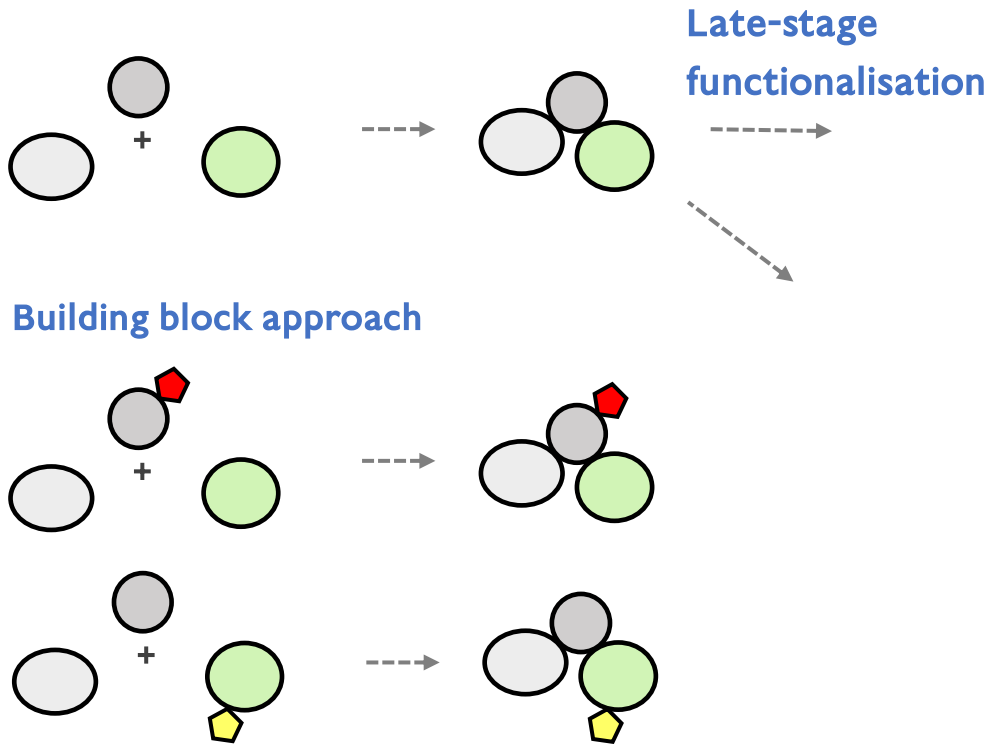


✓ Complex structure (stereochemistry)

✘ Limited amount of starting material

✘ Limited type/position of substituents

Total-Synthesis Approach



✓ Diverse substitution pattern

✓ Suitable for scale-up

✘ Difficult for complex structure

Drug Design and Synthesis – Real Example

Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

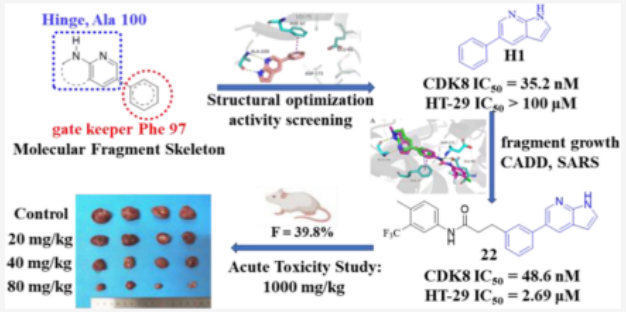
Discovery of the Novel 1*H*-Pyrrolo[2,3-*b*]pyridine Derivative as a Potent Type II CDK8 Inhibitor against Colorectal Cancer

Xing Xing Zhang,† Yun Xiao,† Yao Yao Yan, Yu Meng Wang, Han Jiang, Lei Wu, Jing-bo Shi,* and Xin Hua Liu*

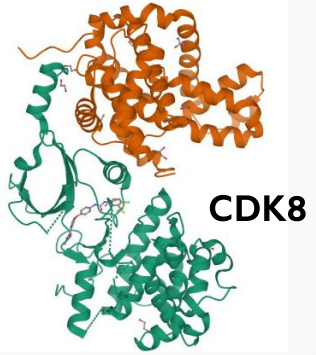
Cite This: *J. Med. Chem.* 2022, 65, 12095−12123 Read Online

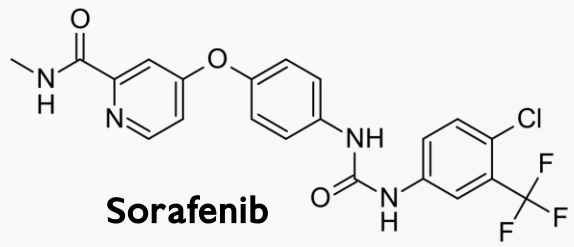
ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Few targeted drugs were approved for treatment of colorectal cancer (CRC). Cyclin-dependent kinase 8 played a vital role in regulating transcription and was a key colorectal oncogene associated to colorectal cancer. Here, through de novo drug design and in depth structure−activity relationship analysis, title compound **22**, (3-(3-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(4-methyl-3-(trifluoromethyl)phenyl)propenamide), was discovered as a potent type II CDK8 inhibitor, which exhibited potent kinase activity with an IC_{50} value of 48.6 nM and could significantly inhibit tumor growth in xenografts of CRC in vivo. Further mechanism studies indicated that it could target CDK8 to indirectly inhibit *β*-catenin activity, which caused downregulation of the WNT/*β*-catenin signal and inducing cell cycle arrest in G2/M and S phases. More importantly, the title compound exhibited low toxicity with good bioavailability (*F* = 39.8%). These results could provide the reference for design of new type II CDK8 inhibitors against colorectal cancer.



J. Med. Chem. 2022, 65, 12095−12123





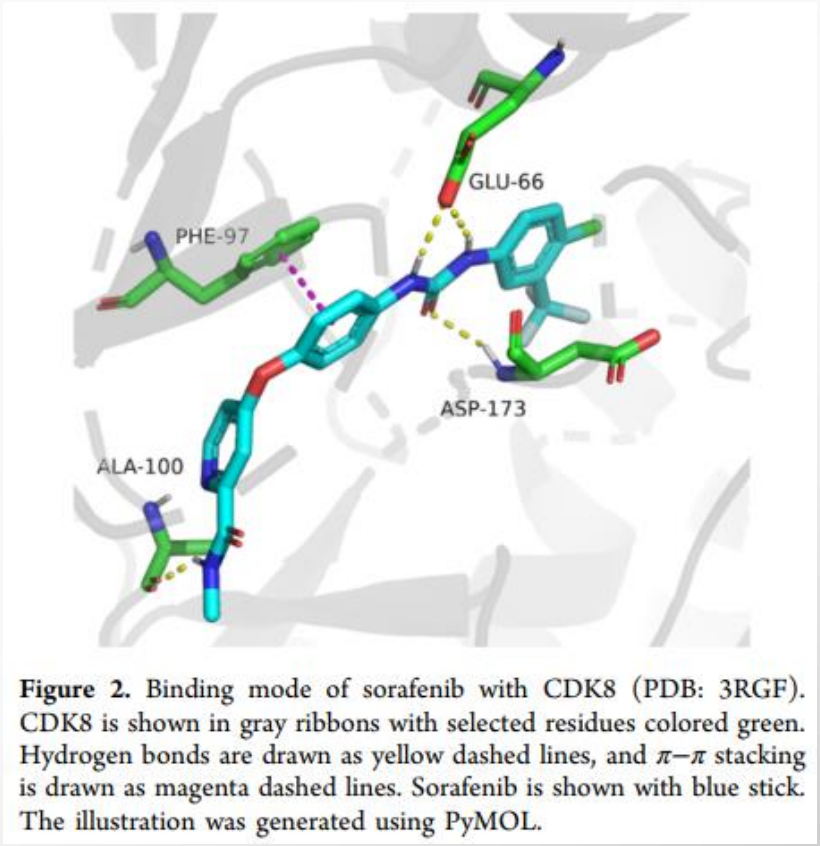


Figure 2. Binding mode of sorafenib with CDK8 (PDB: 3RGF). CDK8 is shown in gray ribbons with selected residues colored green. Hydrogen bonds are drawn as yellow dashed lines, and *π*−*π* stacking is drawn as magenta dashed lines. Sorafenib is shown with blue stick. The illustration was generated using PyMOL.

Drug Design and Synthesis – Real Example

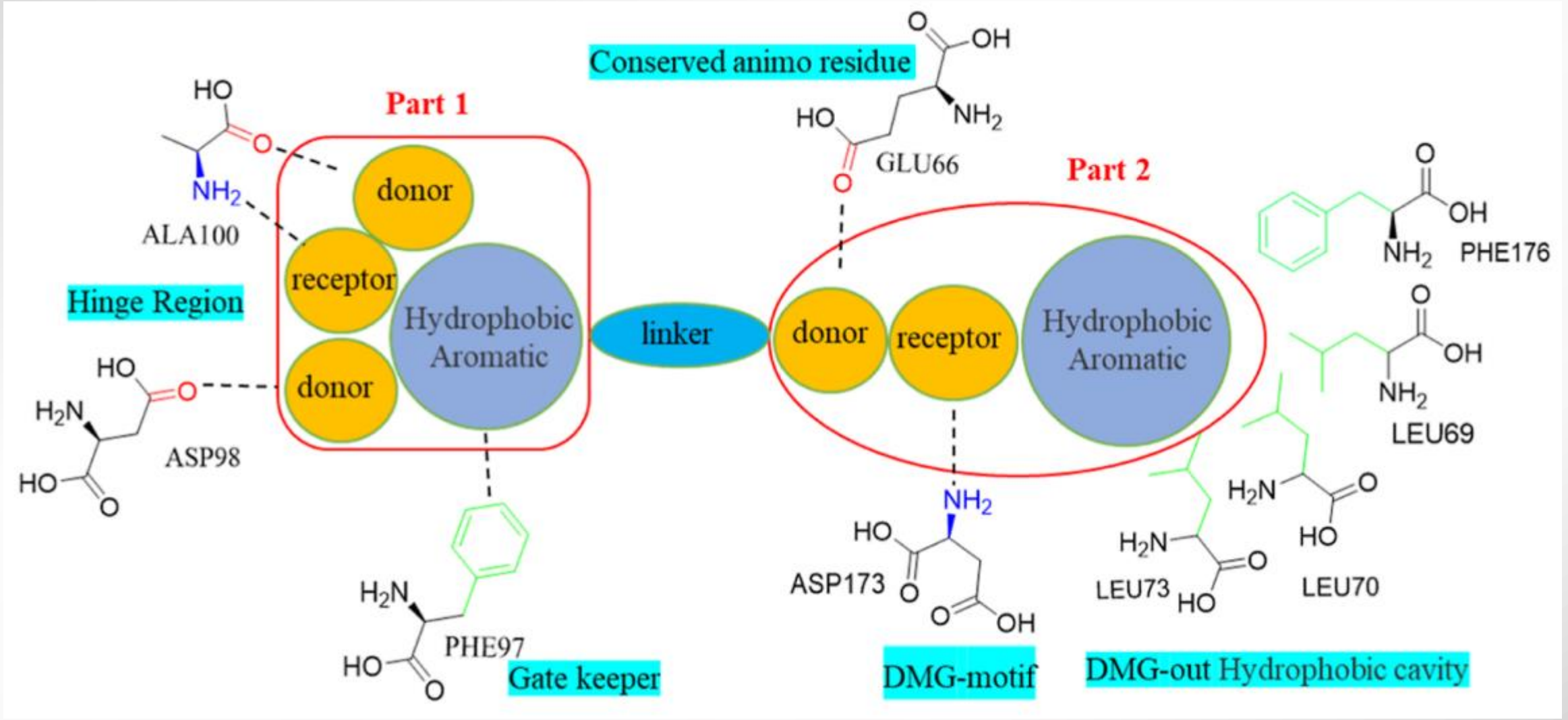


Figure 3. Structural features of ligands and analysis of their interactions.

J. Med. Chem. 2022, 65, 12095–12123

Drug Design and Synthesis – Real Example

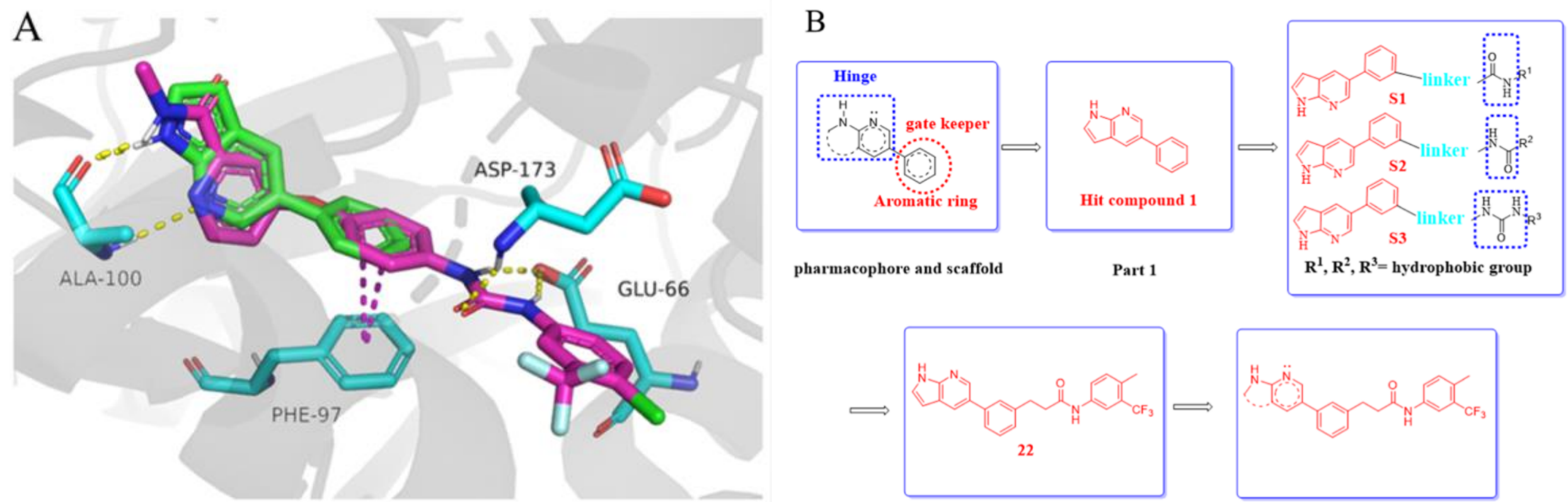
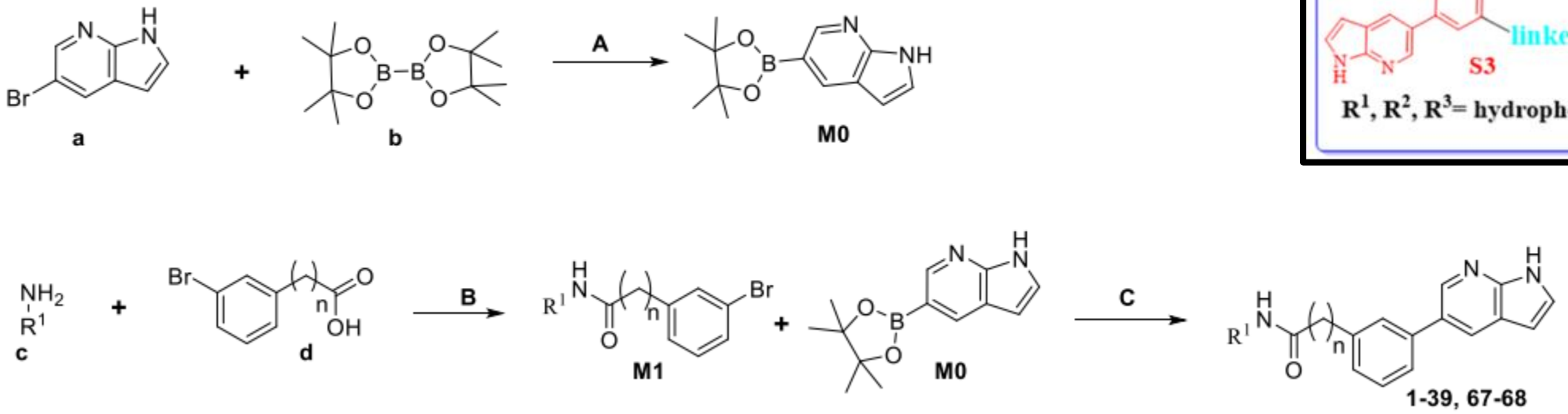


Figure 4. Design idea of title compounds. (A) Superposition of spatial structures of compound **H1** and sorafenib within active sites of CDK8 (PDB: 3RGF). CDK8 is shown in gray ribbons with selected residues colored blue. Hydrogen bonds are drawn as yellow dashed lines, and $\pi-\pi$ stacking is drawn as magenta dashed lines. Compound **H1** is shown with a green stick, and sorafenib is shown with a hot pink stick. The illustration was generated using PyMOL. (B) Design idea.

J. Med. Chem. 2022, 65, 12095–12123

Drug Design and Synthesis – Real Example

Scheme 1. Synthesis of Compounds 1–39 and 67–68[a]

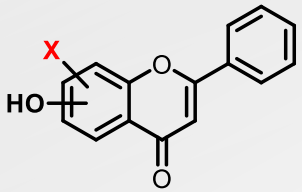


[a]Reagents and conditions: (A) CH_3COOK, $Pd(dppf)Cl_2$, DMF, 78 °C, 12 h; (B) HATU, DIPEA, CH_2Cl_2, 25 °C; (C) K_2CO_3, $Pd(dppf)Cl_2$, 1,4-dioxane, H_2O, 85 °C, 14 h.

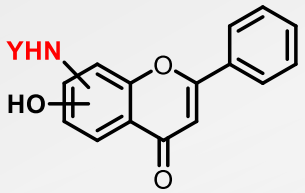
J. Med. Chem. 2022, 65, 12095–12123

Hit to Lead & Lead Optimization

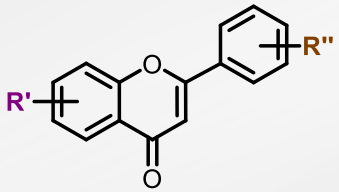
Drug Analogs



X = F, Cl, Br, I, NO_2

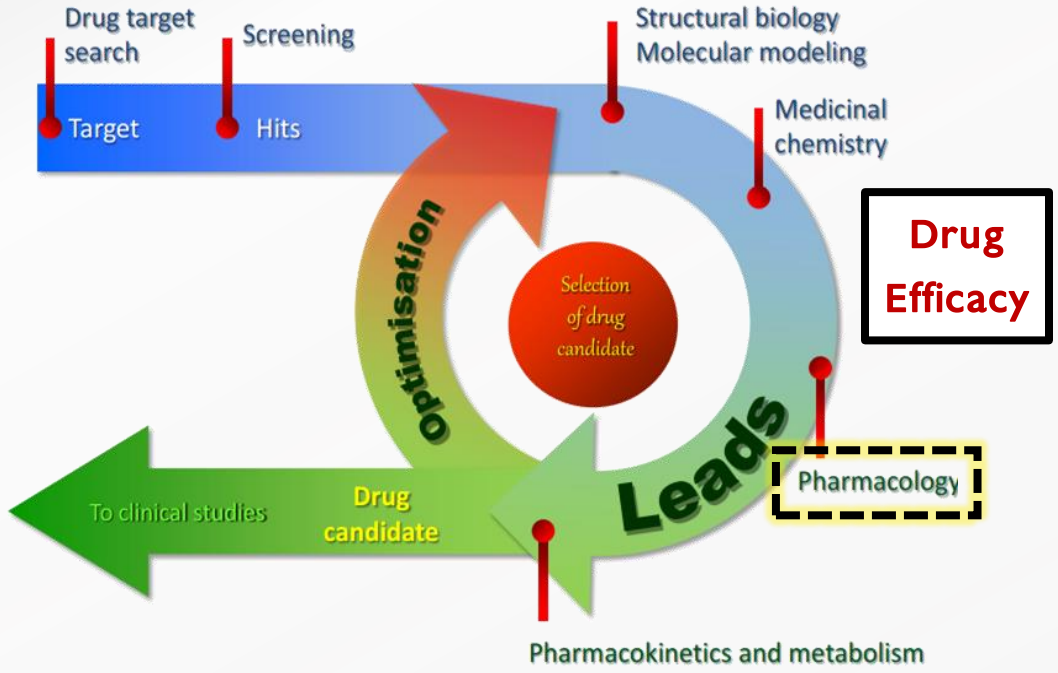


Y = Alkyl, Acyl

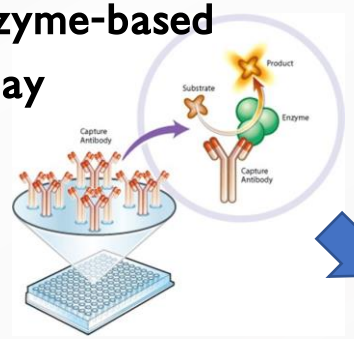


R' = F, Cl, Br, I, NO_2, OMe at 6, 7, 8 or 9

R'' = F, Cl, Br, I, NO_2, OMe at 2', 3' or 4'

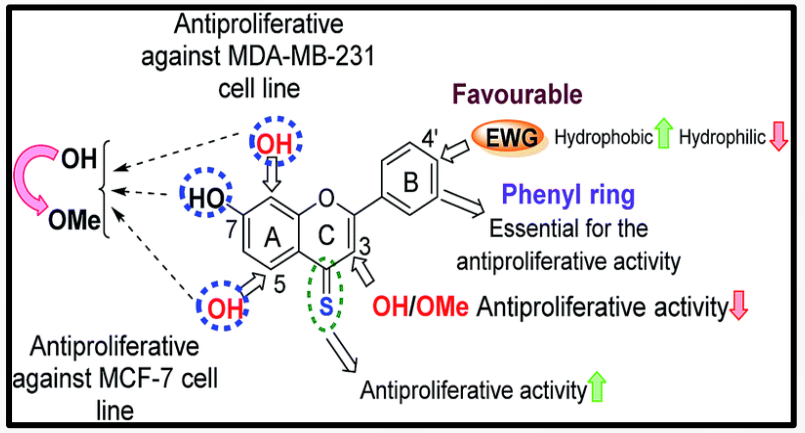


Enzyme-based assay

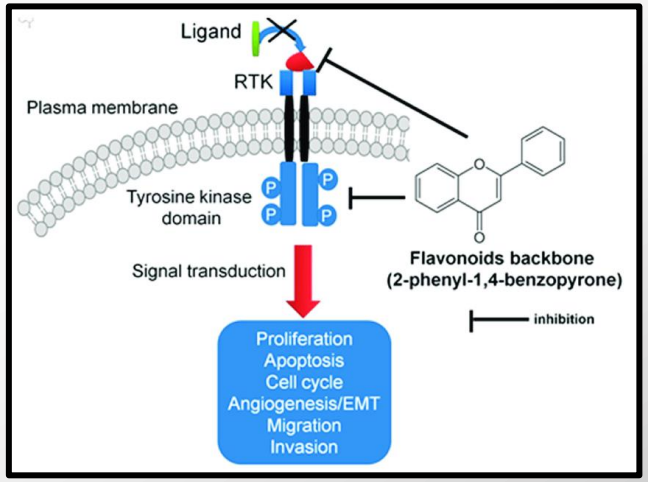


Cell-based assay

In vivo screening



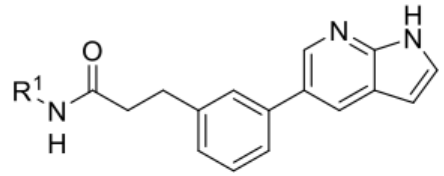
Structure-Activity Relationship

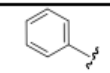
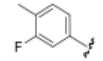
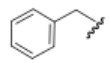
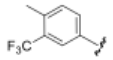
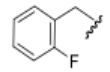
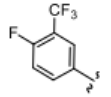
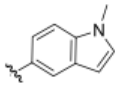
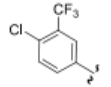
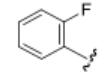
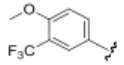
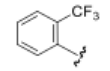
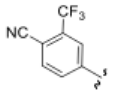
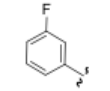
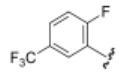


Mechanism of Action

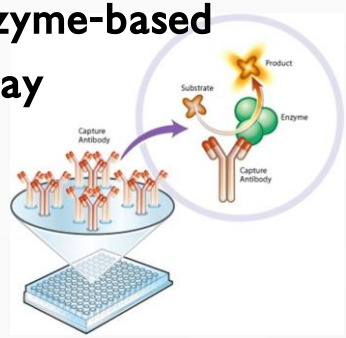
Evaluation of Biological Activity – Real Example

Table 1. CDK8 Inhibition Rate of Compounds 1–39 at 200 nM



Compounds	R^1	Inhibition rate @200 nM[a]	Compounds	R^1	Inhibition rate @200 nM[a]
1		-[b]	21		57.4%
2		4.1%	22		74.3%
3		-[b]	23		24.6%
4		22.2%	24		46.5%
5		28.7%	25		41.4%
6		-[b]	26		58.8%
7		-[b]	27		30.4%

Enzyme-based assay



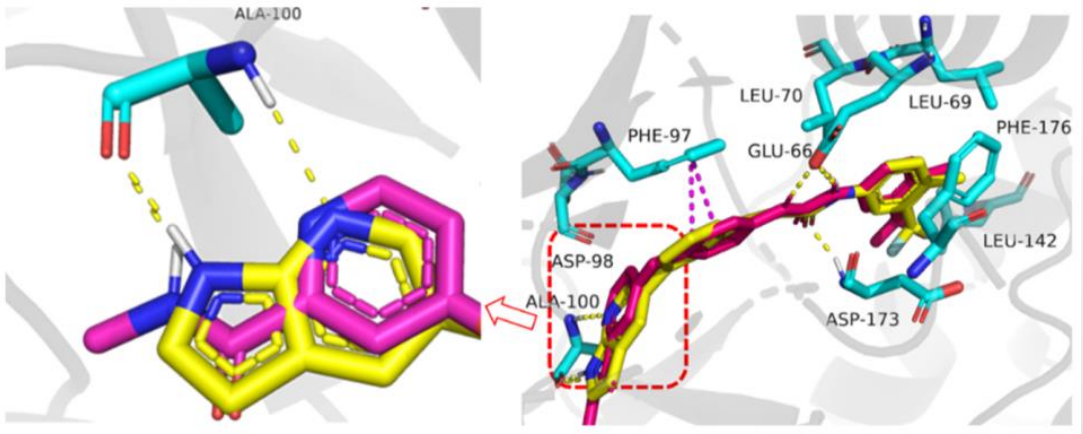


Figure 5. Binding mode of compounds with active sites of CDK8 (PDB: 3RGF). CDK8 is shown in gray ribbons with selected residues colored blue. Hydrogen bonds are drawn as yellow dashed lines, and $\pi-\pi$ stacking is drawn as magenta dashed lines. Compound **22** is shown with a yellow stick, and sorafenib is shown with a hot pink stick. The illustration was generated using PyMOL.

J. Med. Chem. 2022, 65, 12095–12123

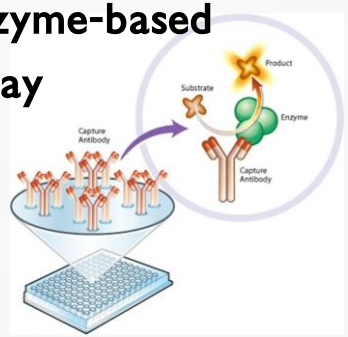
Evaluation of Biological Activity – Real Example

Table 8. Antiproliferative Activity and Preliminary Safety of Selected Compounds

compounds	GI_{50} (μM)[c] HCT-116	HT-29	SW480	CT-26	GES-1	inhibition rate at 200 nM[a] (%)	CDK8 IC_{50} (nM)[b]
22	4.9 ± 1.6	4.3 ± 2.0	2.1 ± 1.3	4.0 ± 0.8	61.5 ± 4.0	74.3	46.5 ± 1.5
17	73.7 ± 4.2	>100	>100	>100	>100	45.9	187.6 ± 2.0
18	62.5 ± 2.3	27.3 ± 3.1	46.1 ± 2.5	>100	>100	44.9	190.7 ± 1.3
21	>100	>100	>100	>100	>100	57.4	158.4 ± 1.5
24	7.3 ± 1.7	5.5 ± 1.2	8.3 ± 2.0	10.9 ± 0.9	53.4 ± 2.1	46.5	177.2 ± 1.2
26	10.4 ± 3.1	>100	>100	22.5 ± 1.9	13.8 ± 2.4	58.8	125.9 ± 1.4
29	22.8 ± 4.8	>100	>100	30.2 ± 2.4	18.1 ± 1.8	54.5	143.3 ± 1.7
33	>100	>100	>100	>100	>100	69.2	62.8 ± 1.5
38	82.5 ± 3.4	>100	5.9 ± 0.9	7.7 ± 1.0	75.5 ± 2.1	68.5	64.5 ± 1.8
50	50.1 ± 3.6	15.5 ± 1.9	>100	35.5 ± 3.3	>100	64.7	70.4 ± 1.4
68	>100	>100	>100	>100	>100	70.4	55.1 ± 2.1
69	20.2 ± 2.7	44.5 ± 2.4	>100	>100	38.8 ± 3.4	51.6	169.8 ± 1.7
70	17.2 ± 2.9	10.5 ± 1.1	>100	5.0 ± 0.8	98.4 ± 4.2	47.7	185.2 ± 1.6
71	39.5 ± 3.1	20.7 ± 1.4	31.5 ± 1.9	10.6 ± 0.9	50.7 ± 3.0	42.3	225.3 ± 1.8
72	27.5 ± 1.6	22.3 ± 3.2	21.1 ± 0.9	15.4 ± 1.8	83.2 ± 1.7	62.1	85.4 ± 1.5
73	17.2 ± 2.0	14.9 ± 1.8	47.3 ± 3.1	15.5 ± 2.0	22.1 ± 1.9	69.5	53.9 ± 1.6
74	18.5 ± 1.8	22.4 ± 1.7	52.4 ± 3.5	19.0 ± 2.1	>100	46.1	197.2 ± 2.1
75	14.3 ± 2.5	25.5 ± 3.0	>100	8.0 ± 0.9	20.1 ± 0.6	50.0	185.4 ± 1.4
76	>100	6.0 ± 0.6	12.2 ± 1.5	22.7 ± 1.6	44.3 ± 2.0	64.2	68.5 ± 1.5
77	22.3 ± 3.1	52.5 ± 3.4	20.8 ± 2.4	14.2 ± 2.9	>100	70.2	40.3 ± 1.7
78	28.5 ± 1.7	30.2 ± 1.4	>100	>100	>100	54.7	167.5 ± 1.8
Sorafenib	9.0 ± 1.8	25.4 ± 1.7	15.6 ± 3.2	14.7 ± 1.7	15.9 ± 3.0	71.1	71.5 ± 2.0
CCT-251545	9.5 ± 0.8	5.8 ± 1.0	1.8 ± 0.4	11.2 ± 1.6	50.0 ± 1.1	82.9	17.9 ± 1.5
SEL120-34A	11.3 ± 1.9	33.8 ± 1.4	17.7 ± 2.1	53.9 ± 1.9	55.3 ± 3.2	75.6	37.2 ± 2.5

[a]Inhibition rate (%) on CDK8 enzyme activity of compounds at 200 nM. Values from a dependent experiment. [b]C_{50} values were determined by CDK8 enzyme activity assay. [c]GI_{50} values were determined by MTT assay.

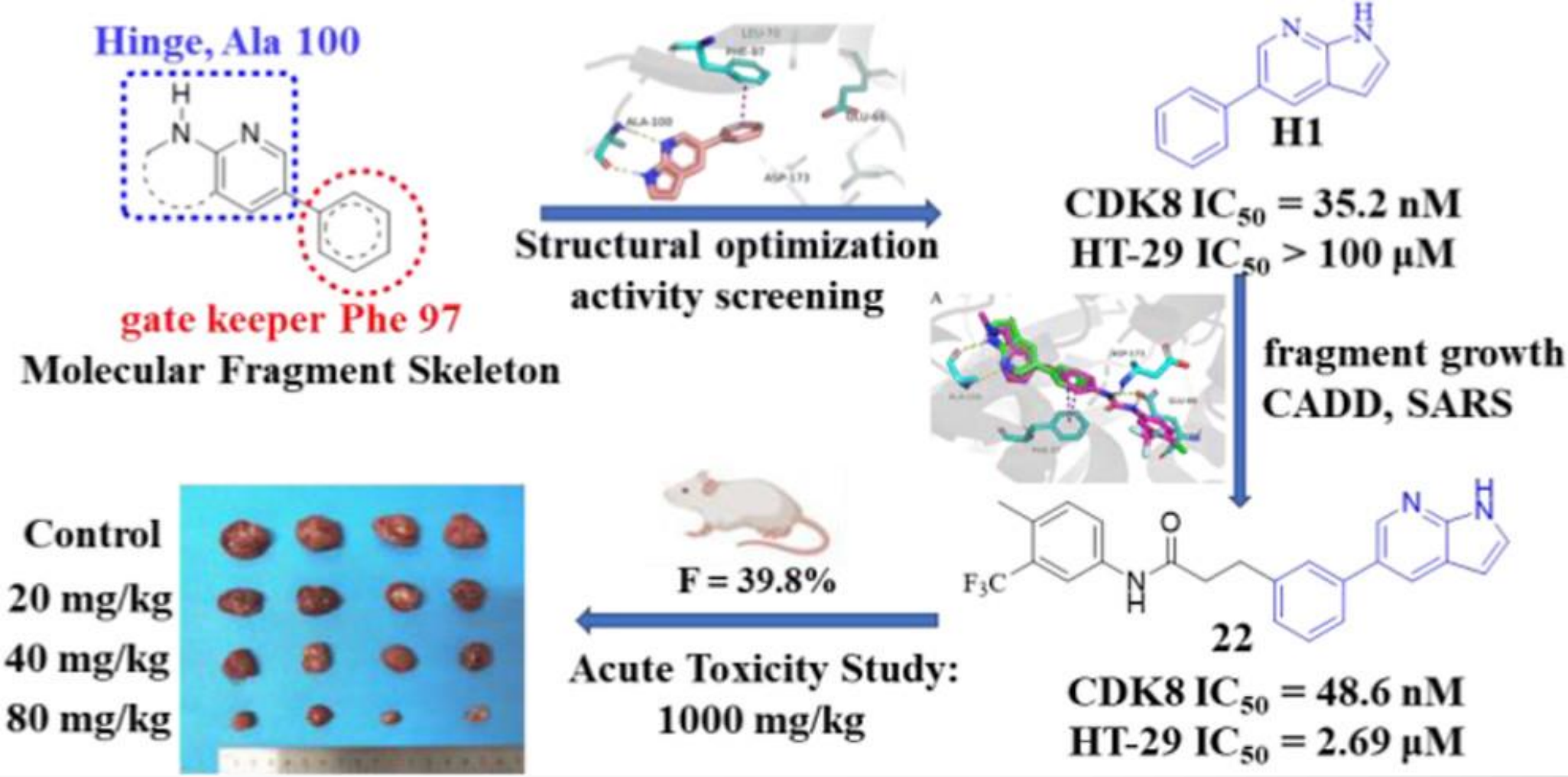
Enzyme-based assay



Cell-based assay

J. Med. Chem. 2022, 65, 12095–12123

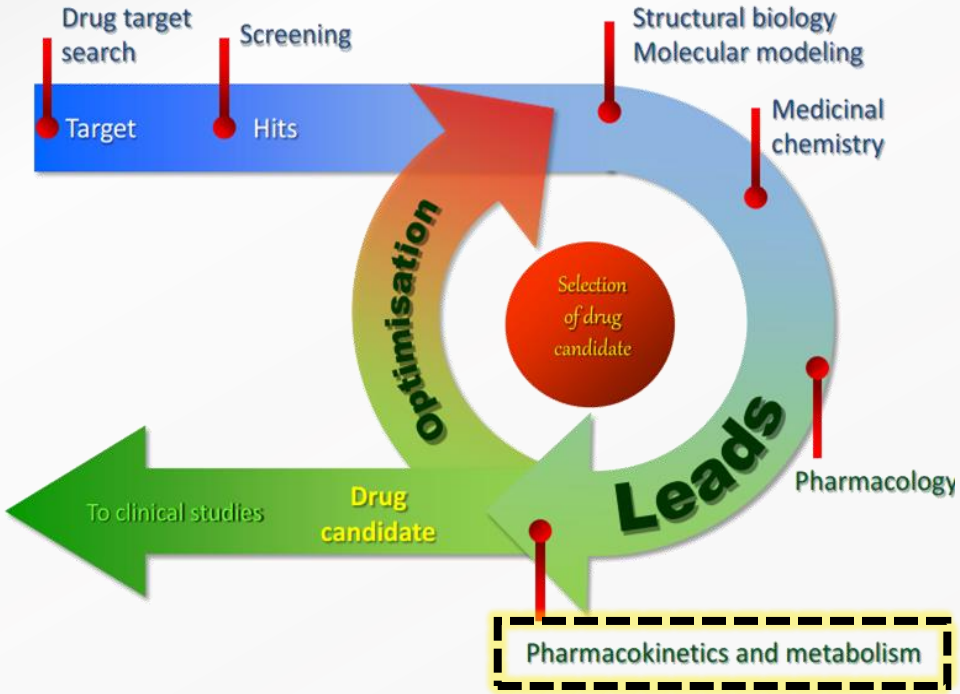
Evaluation of Biological Activity – Real Example

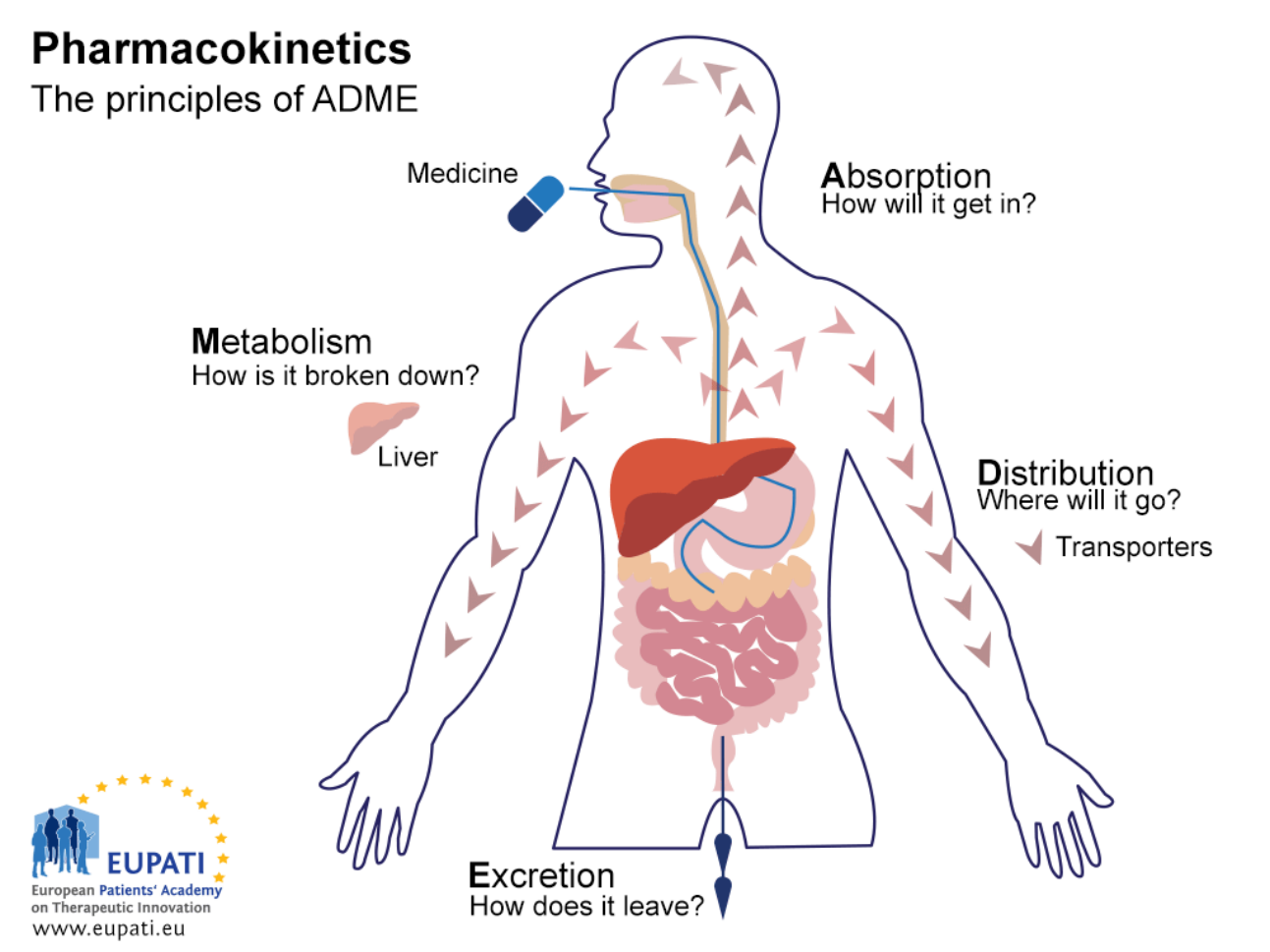


J. Med. Chem. 2022, 65, 12095–12123

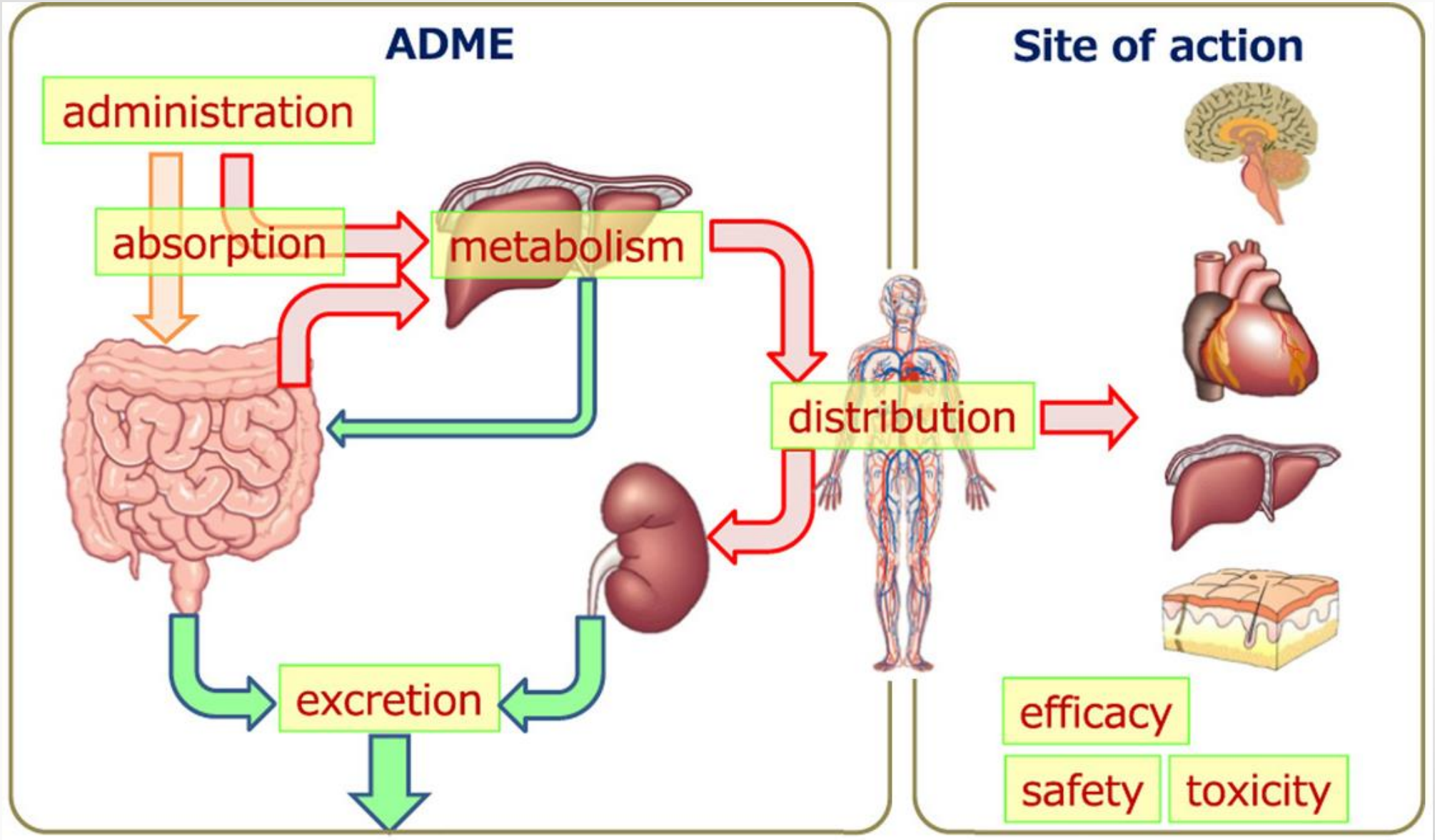
Hit to Lead & Lead Optimization

- Physicochemical Properties & Solubility
- Pharmacokinetics - Adsorption, Distribution, Metabolism and Excretion (ADME) properties
- Toxicity potential

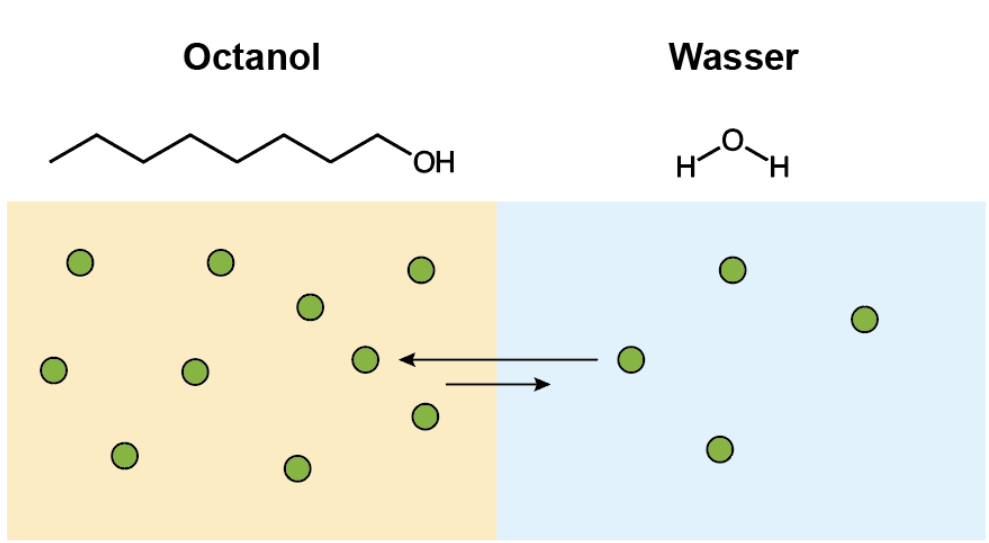




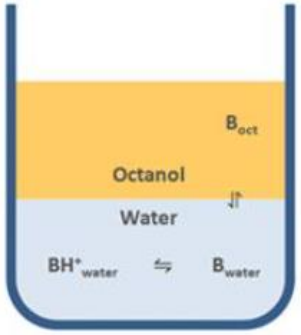
Pharmacokinetics – ADMET properties

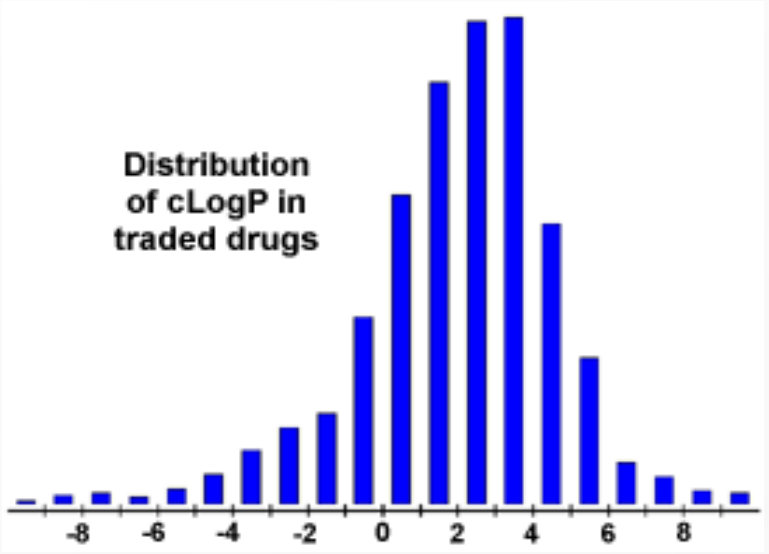


Lipophilicity and Solubility

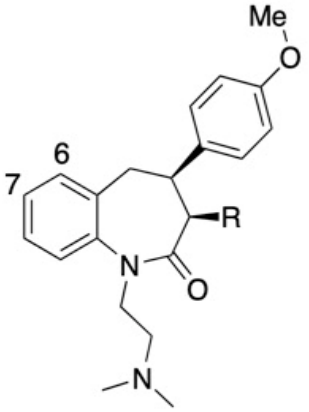


$$\log P = \log \frac{C\ (\text{Octanol})}{C\ (\text{Wasser})}$$

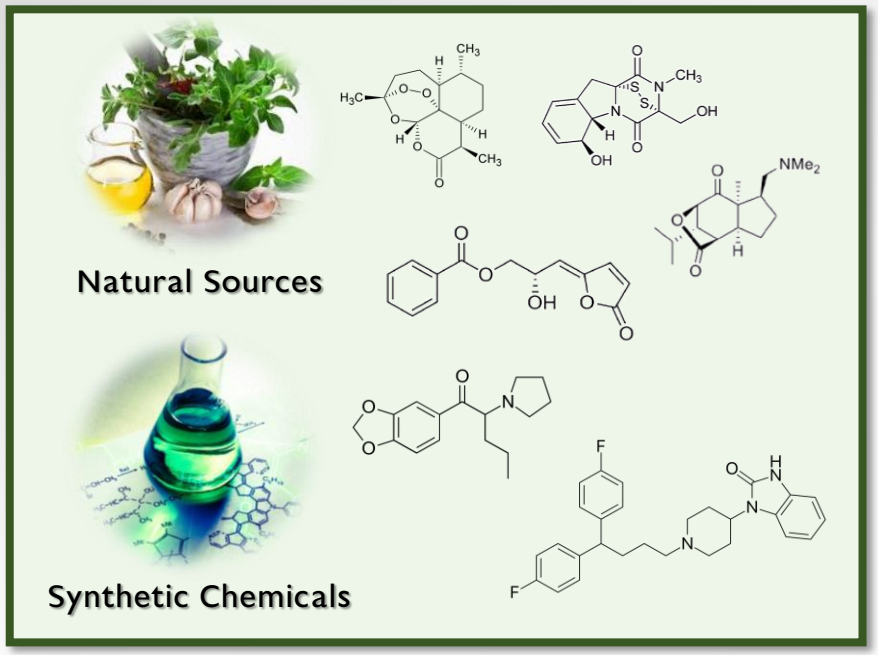




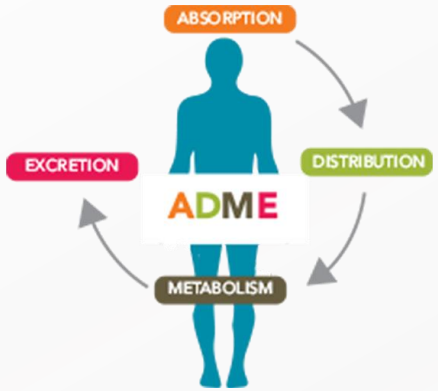
X	R	IC_{50} (μM)	logP
H	$OCOCH_3$	4.7	1.2
6-Cl	$OCOCH_3$	1.6	2.4
6-CH_3	$OCOCH_3$	2.5	1.7
6-CN	$OCOCH_3$	0.12	1.3
6-CF_3	$OCOCH_3$	0.15	2.9
6-OMe	$OCOCH_3$	2.4	2.4
6-$CONH_2$	$OCOCH_3$	30	-1.0
6-OCH_3,7-Br	CH_3	0.085	3.4
7-OC_6H_5	$OCOCH_3$	0.44	3.1
7-$OCONHCH_3$	$OCOCH_3$	12	0.31
7-CF_3	CH_3	0.076	3.6

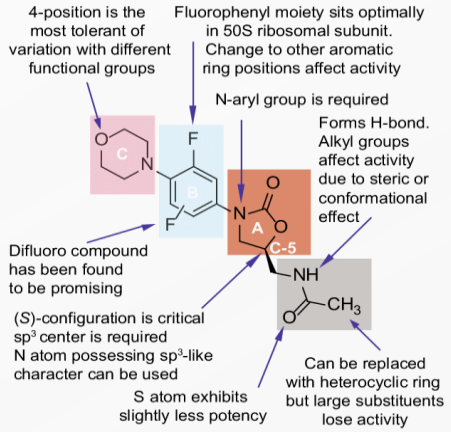


Research in the Field of Drug Discovery



Library

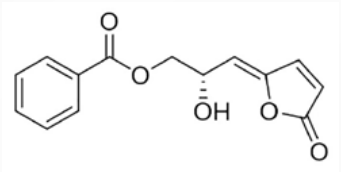


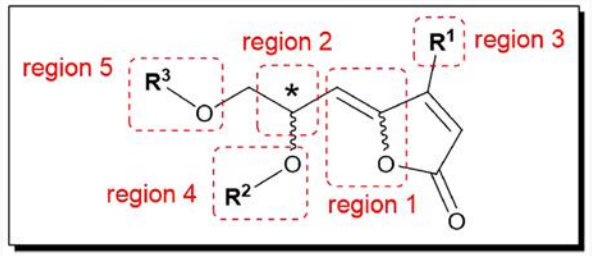


Structure-Activity Relationship

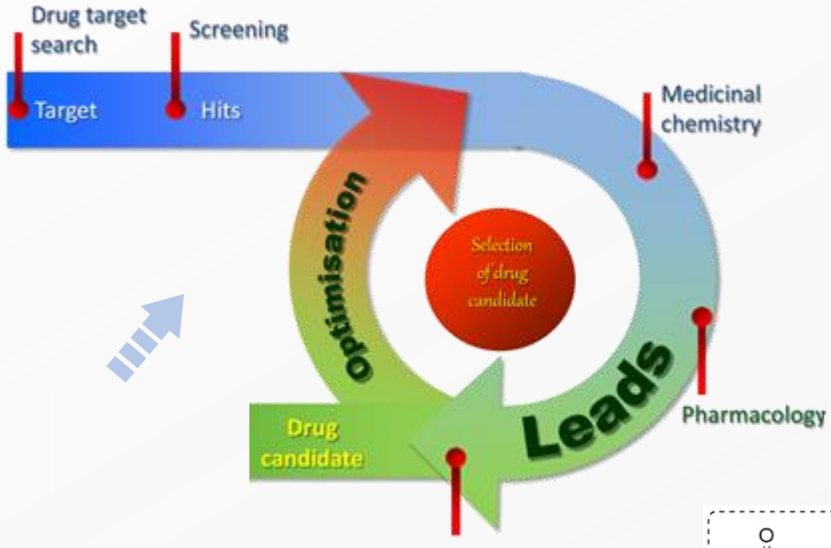
Biological Activity

Hits

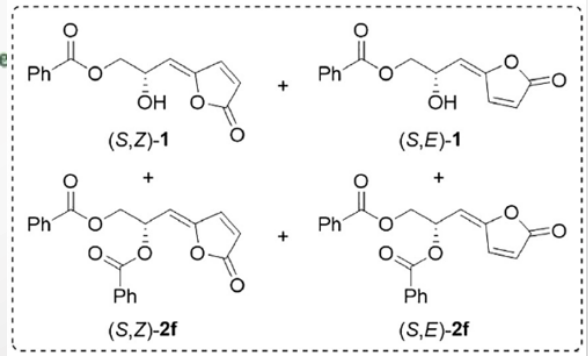




Drug Design



Organic Synthesis



Drug analogues