University of Silesia, Katowice, Poland
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Strategies in drug discovery

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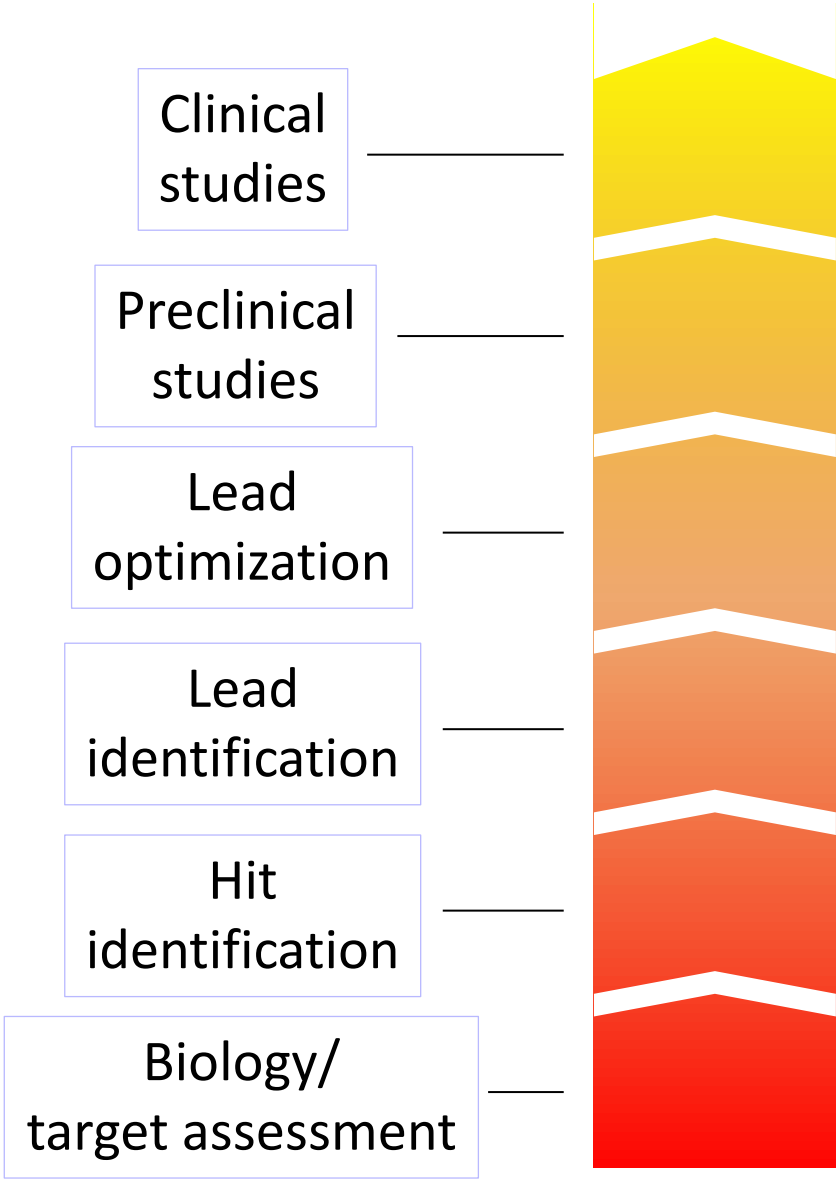
Drug development



Hits and leads

Hit:

(1) reproducible activity in a relevant bioassay,

(2) confirmed structure and high purity,

(3) specificity for the target under study,

(4) confirmed potential for novelty

(5) chemically tractable structure, that is, molecules presenting a certain affinity for a target.

Lead:

(1) must be active *in vivo*,

(2) must not display human ether-a-go-go-related (hERG) toxicity,

(3) the analogs of the hit must display clear structure–activity relationships (SAR),

(4) must not contain chemically reactive functions

(5) must provide patent opportunities

Four strategies in “hit” discovery:

I. Bruteforce
(screening)

II. Exploitation of biological information
(traditional medicine, natural products, observation made for humans and animals)

III. Rational approach

IV. By chance

I. Systematic screening: Extensive screening

Discovery of benzodiazepines by Sternbach:

(1) the chemical series had to be relatively unexplored,

(2) it had to be easily accessible,

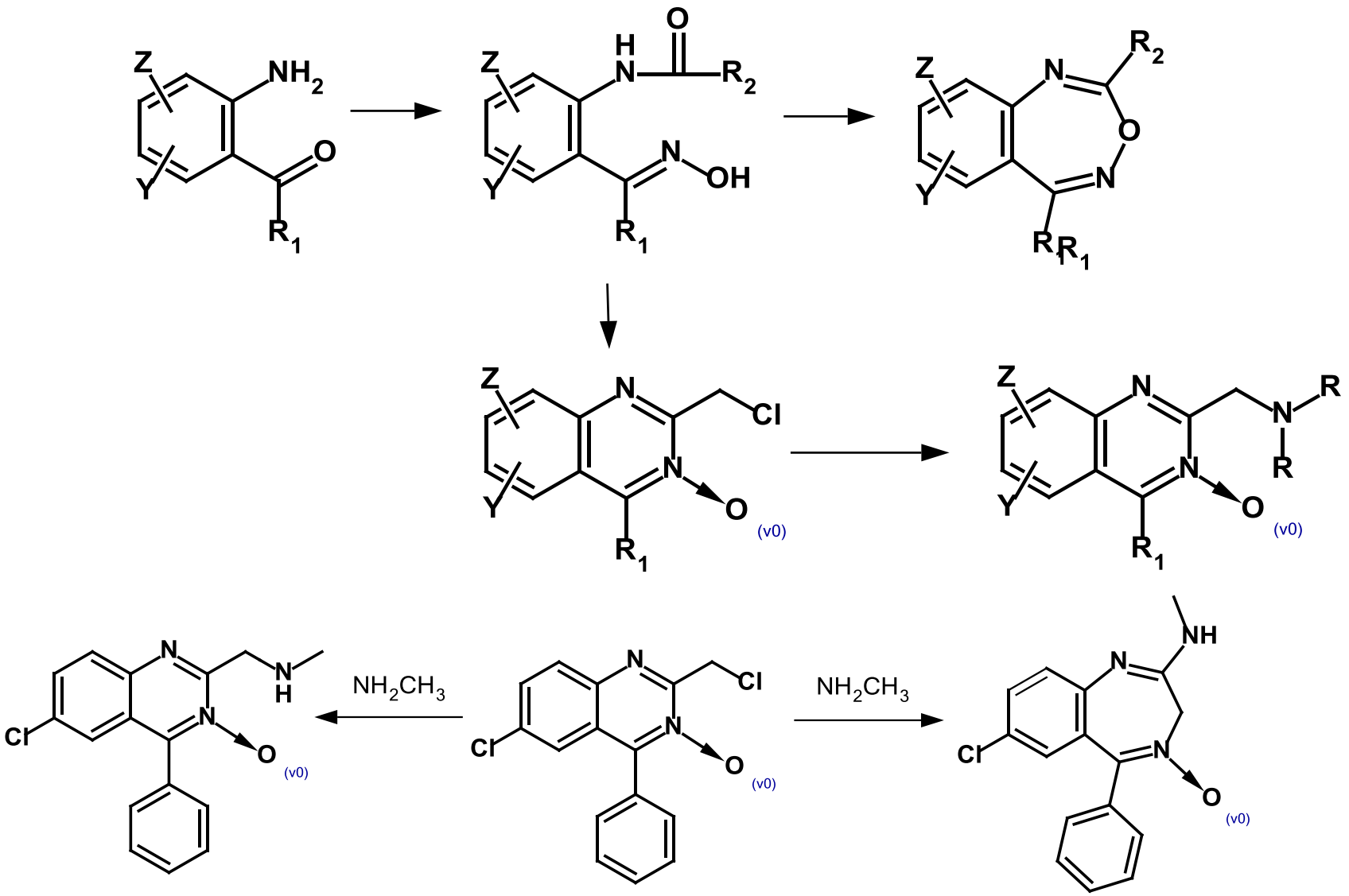
(3) it had to allow a great number of variations and transformations,

(4) it had to offer some challenging chemical problems

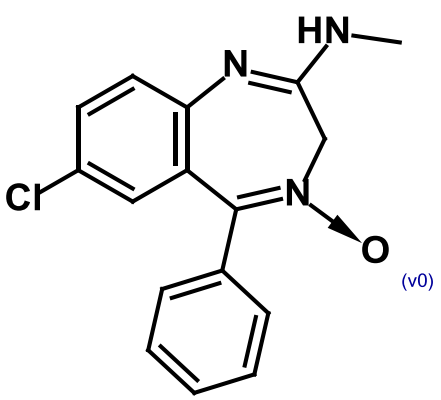
(5) it had to “look” as if it could lead to biologically active products.

Sternbach L.H. The benzodiazepine story. J. Med. Chem. 1979, 22, 1–7

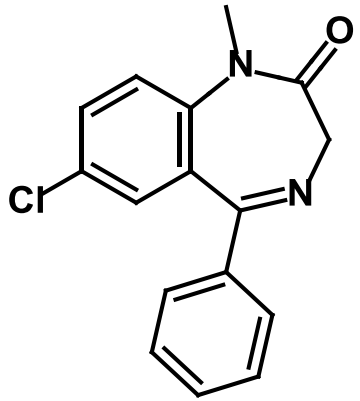
I. Systematic screening: Benzodiazepine discovery



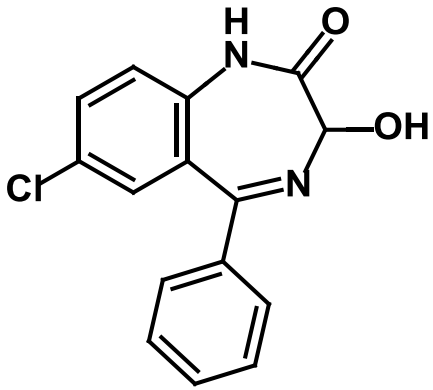
I. Systematic screening: Benzodiazepine discovery



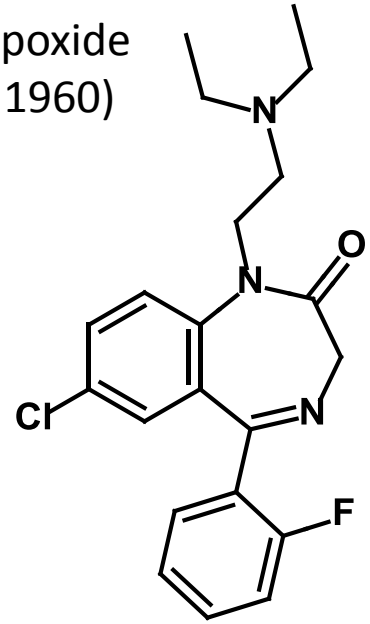
chlordiazepoxide
(Librium, 1960)



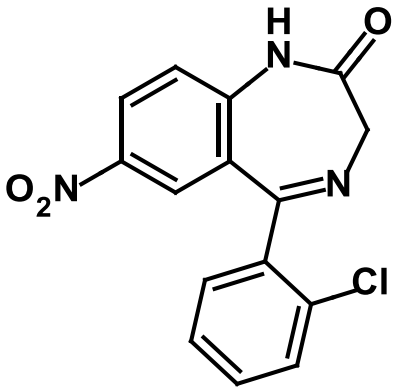
diazepam
(Valium, 1963)



oxazepam
(Serax, 1965)

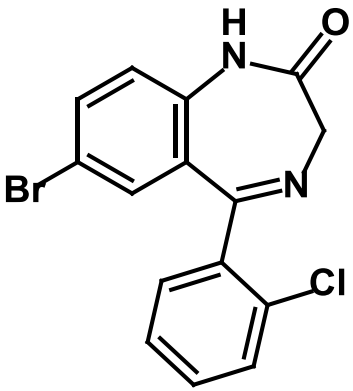


flurazepam
(Dalmane, 1970)

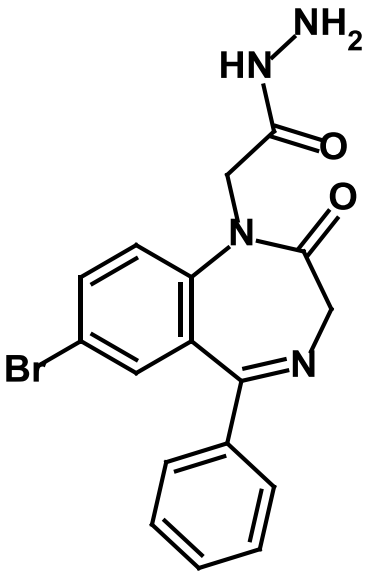


clonazepam
(Clonopin, 1975)

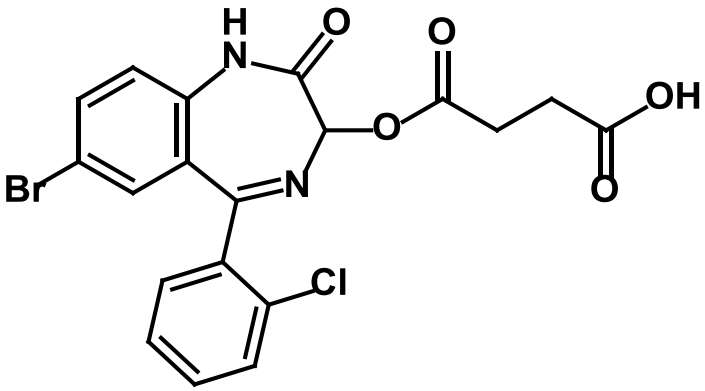
I. Systematic screening: Benzodiazepine discovery



Phenazepam, 1970



Hydazepam, 1984



Cinazepam, 1990
(Levana IC)

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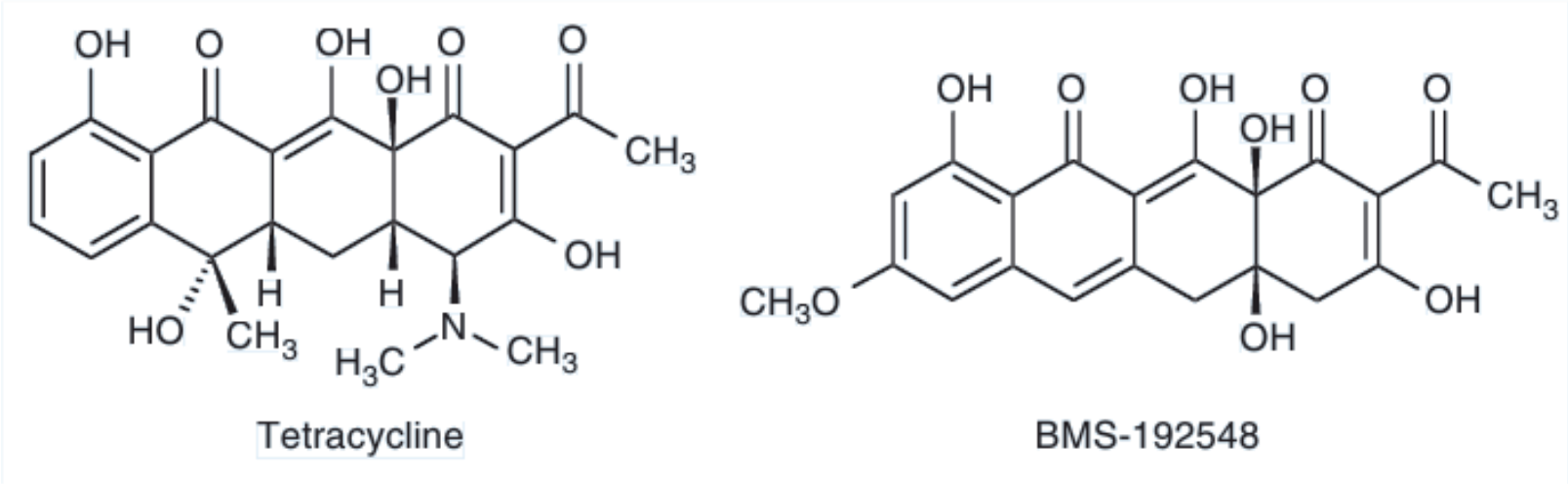
I. Systematic screening: Random screening



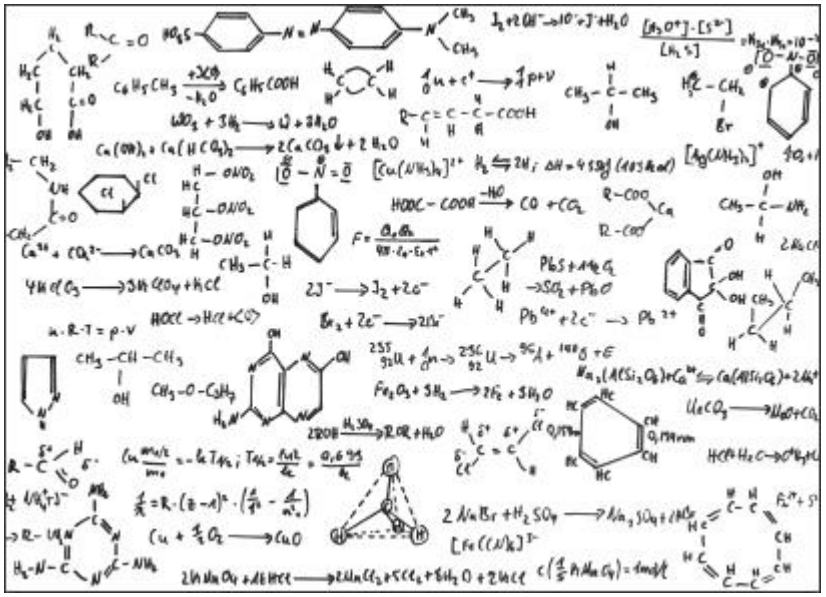
antibiotic

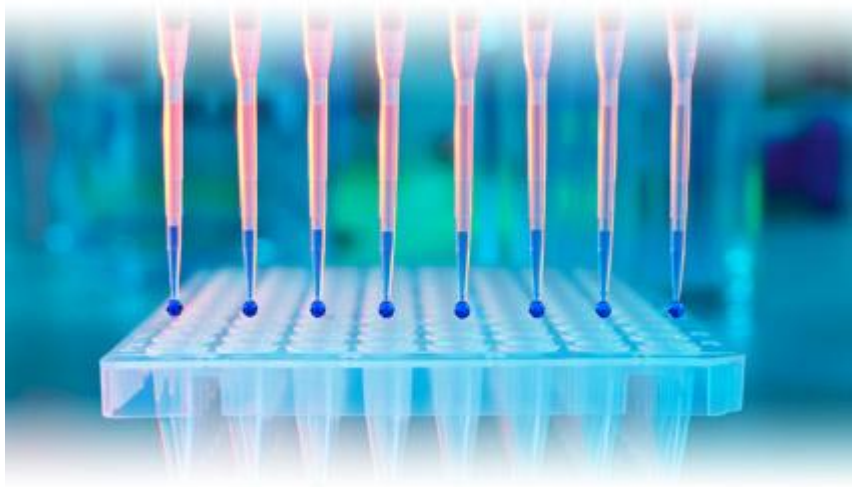
ligand of neuropeptide Y receptor (CNS activity)

- Unexpected activity can be discovered for very similar compounds
- No rational approach (like fishing)
- Test model should be very cheap and effective

I. Systematic screening: High-throughput screening

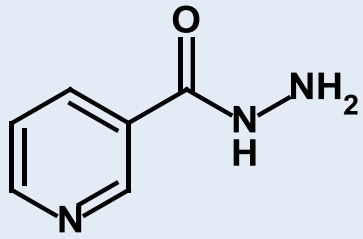
Thousands of molecules × Dozens of assays



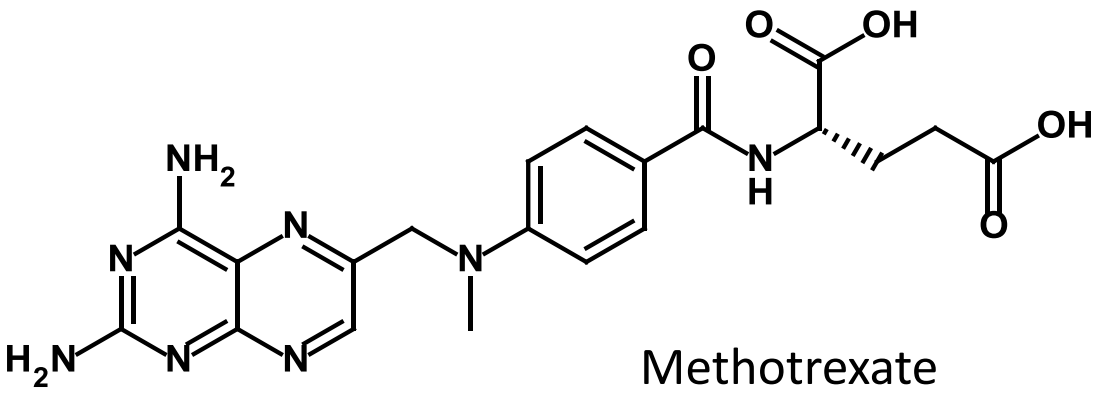


- Very fast and effective
- Need diverse libraries of chemical compounds.

I. Systematic screening: Screening of synthesis intermediates

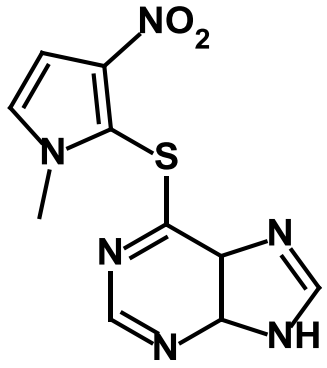


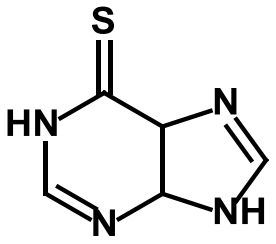
Isoniazid
(tuberculostatic)



Methotrexate
(DHFR inhibitor, leukemia)

Azathioprine
prodrug of mercaptopurine
potent imunosuppressor





Mercaptopurine
(active but toxic)

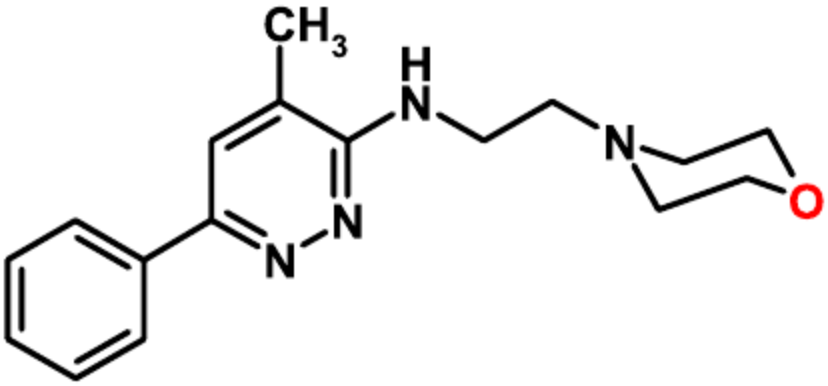
SOSA – **S**elective **O**ptimization of **S**ide **A**ctivities

1. Screening of already known drug molecules with known bioavailability and toxicity on newly identified targets. All found hits will be drug-like molecules by definition!

2. Modification of structures of obtained molecules to optimize target activity and decrease activity for another targets.

Side activity becomes main activity.

I. Systematic screening: SOSA example

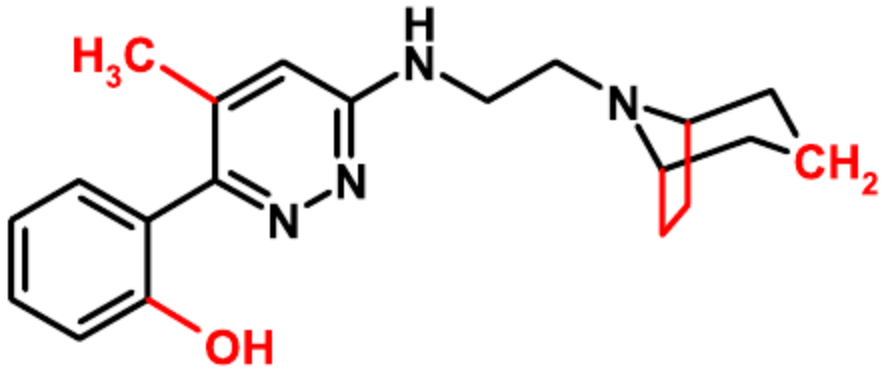


Minaprine (Cantor®)

Dopaminergic: +++

Serotoninergic: ++

Cholinergic: 1/2+



Modified Analogue

Dopaminergic: o

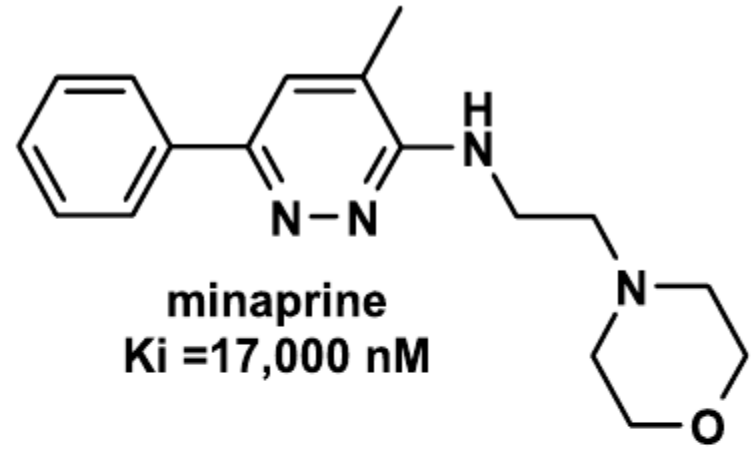
Serotoninergic: o

Cholinergic: ++++

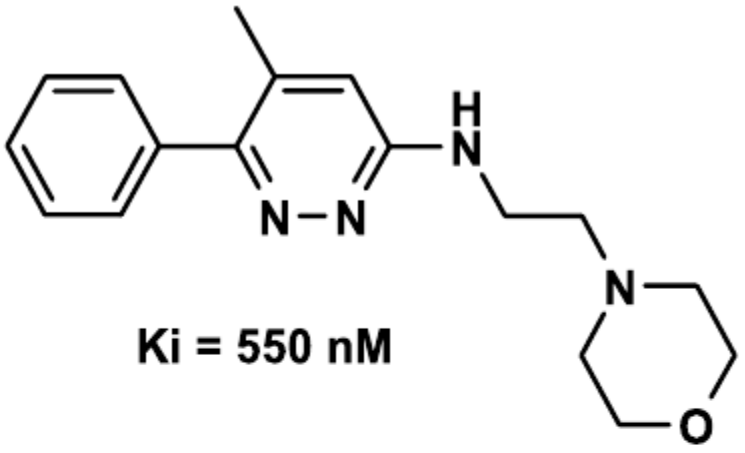
Wermuth, C. G. *Il Farmaco* 1993, 48, 253-274.

I. Systematic screening: SOSA example

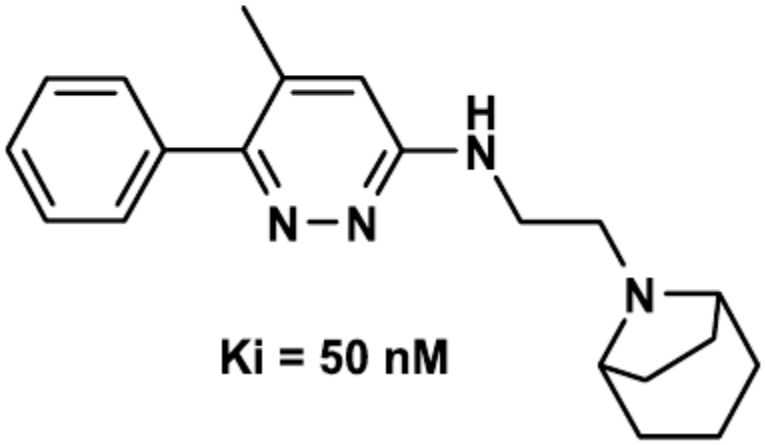
Activity profile inversion



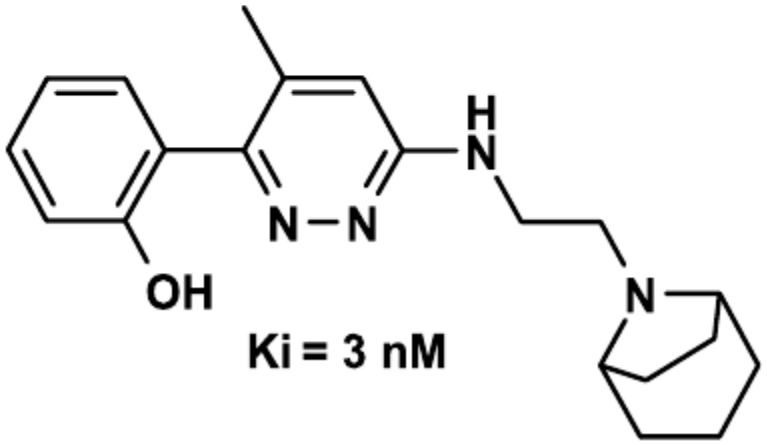
minaprine
Ki =17,000 nM



Ki = 550 nM



Ki = 50 nM



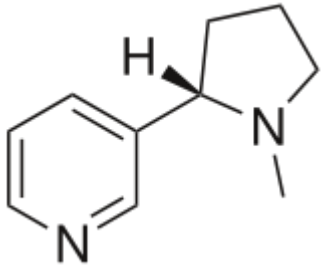
Ki = 3 nM

Wermuth, C. G. *Il Farmaco* 1993, 48, 253-274.

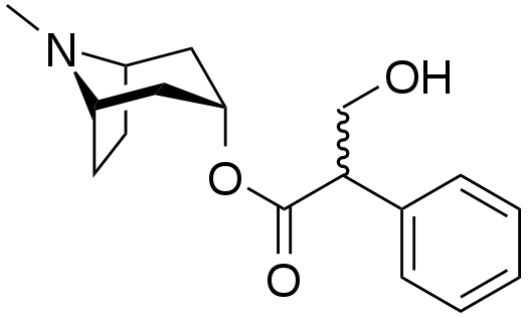
II. Exploitation of biological information

Exploitation of observations made in humans

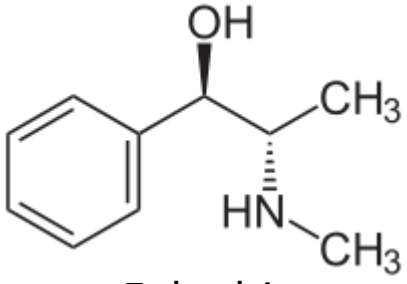
1. Ethnopharmacology



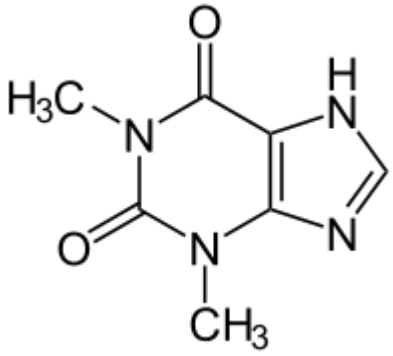
Nicotine
(insecticide, stimulant)
Solanaceae family



Atropine
(antagonist of the muscarinic acetylcholine receptors)
Atropa belladonna, Mandragora officinarum

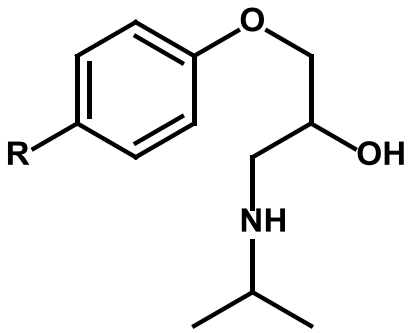


Ephedrine
(stimulant, appetite suppressor, etc)
Ephedra sinica

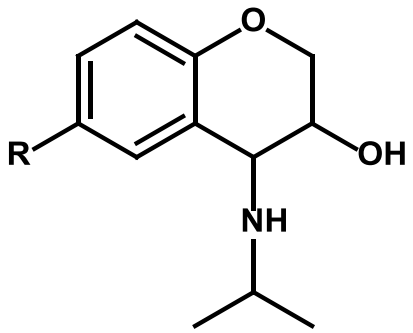


Theophillin
(asthma)
tea, cocoa

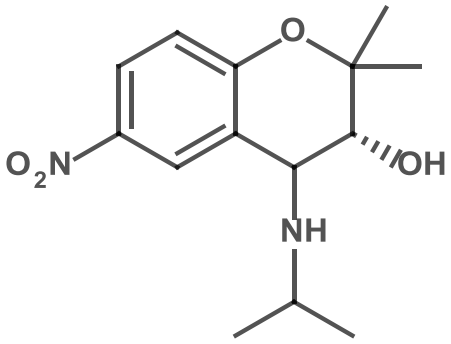
2. Clinical observation of side effects of medicines



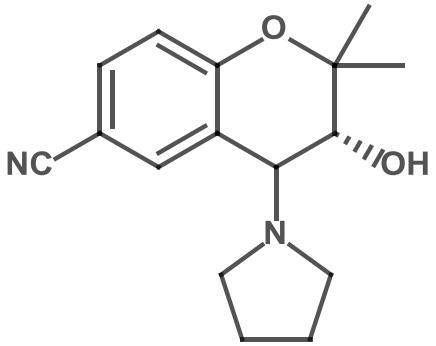
Propanolol
β-adrenergic receptor blocker
(anxiety, panic, hypertension)



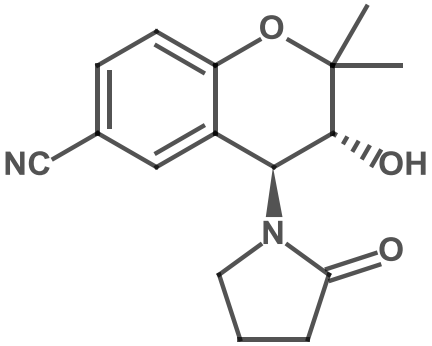
anti-hypertensive activity
no β-blocking activity



1



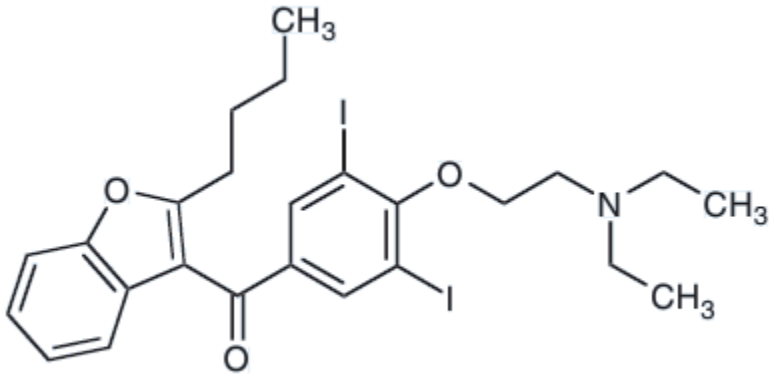
2
(100-fold active than 1)



Cromakalim – active metabolite of 2
(anti-hypertensive)

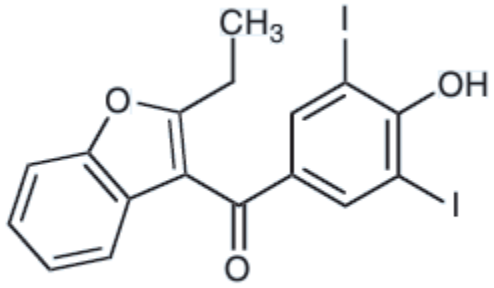
II. Exploitation of biological information

3. New uses for old drugs



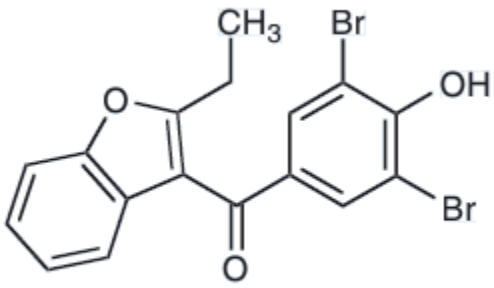
Amiodarone

primary use:
coronary dilator for angina
secondary use:
Wolff-Parkinson-White syndrome



Benziodarone

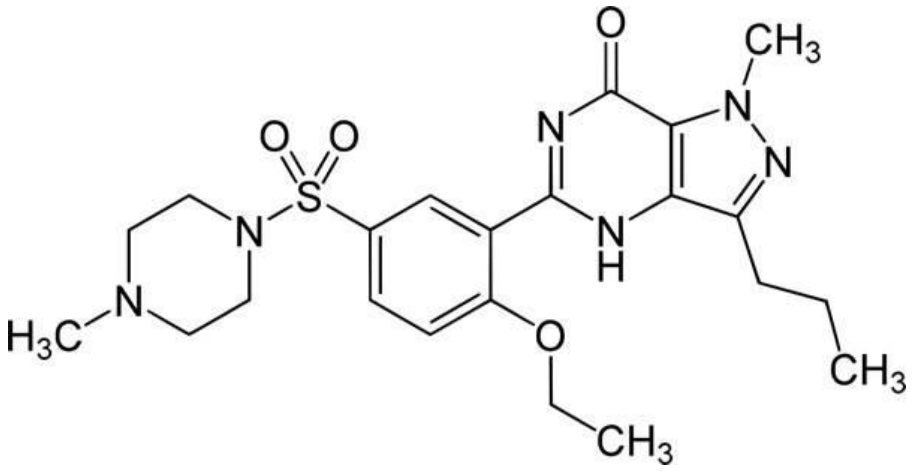
primary use:
coronary dilator
secondary use:
uricosuric agent



Benzbromarone

uricosuric agent

II. Exploitation of biological information



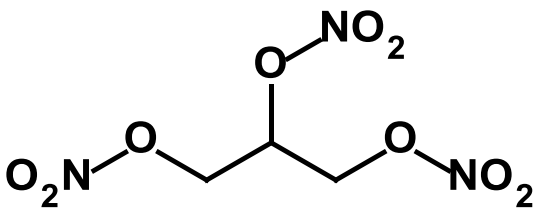
Viagra

primary use: hypotensive and cardiotonic

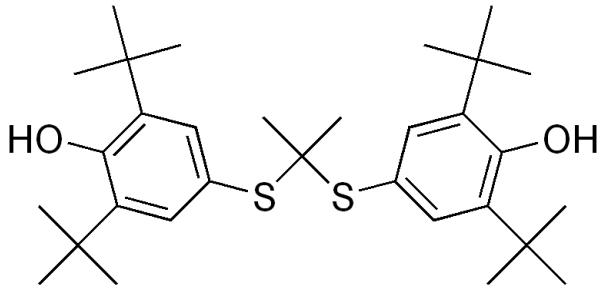
secondary use: male erectile disfunction

- Exploitation of effects which are directly observed on man not on animal models.
- Allow to detect new therapeutic activities even when no pharmacological models in animals do exist

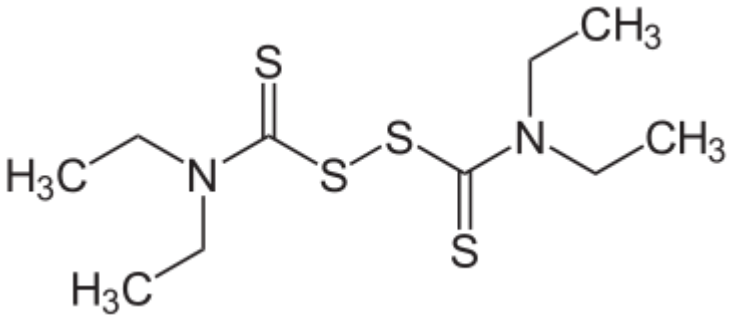
4. Observation from industry chemical products



Nitroglycerin
toxic, but has vasodilating properties

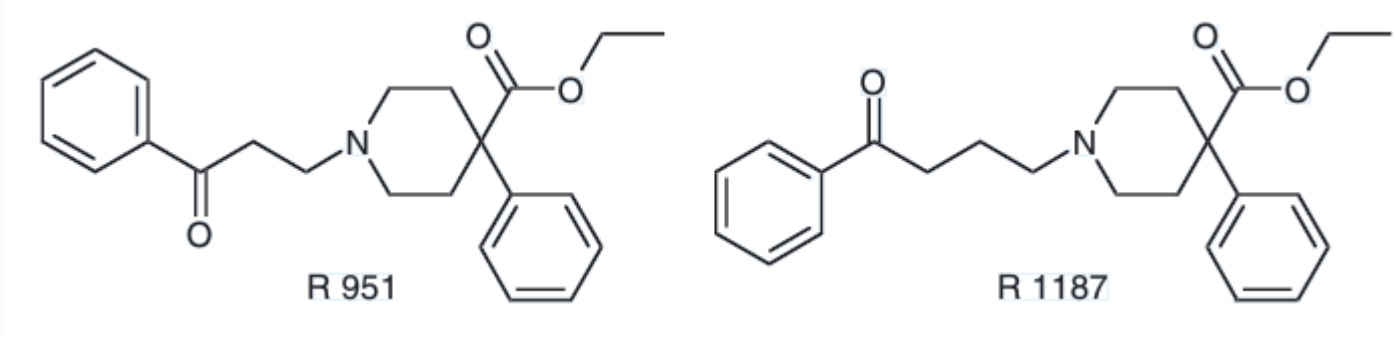


Probucol
anti-hyperlipidemic drug initially used as an antioxidant for plastics and rubber

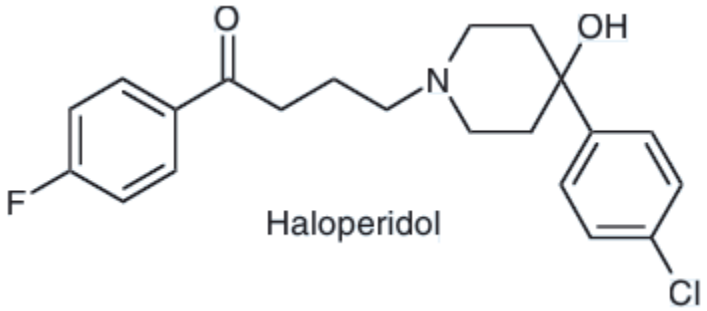


Difulfiram
antioxidant in the rubber industry
alcohol withdrawal treatment

Exploitation of observations made in animals



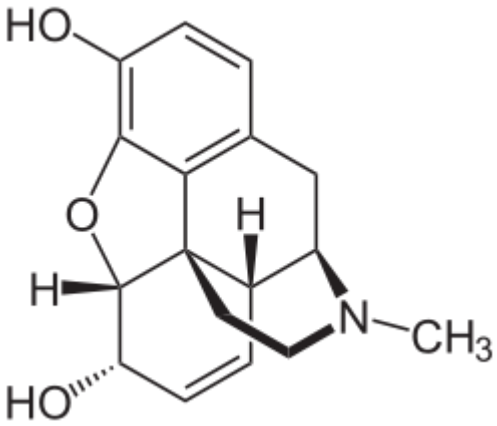
analgetic activity on mice model



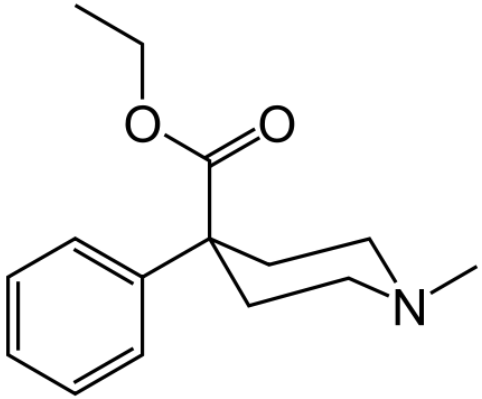
the most potent tranquillizer

III. Rational drug design

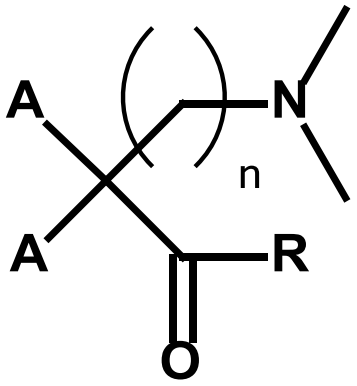
Example: analgetics development



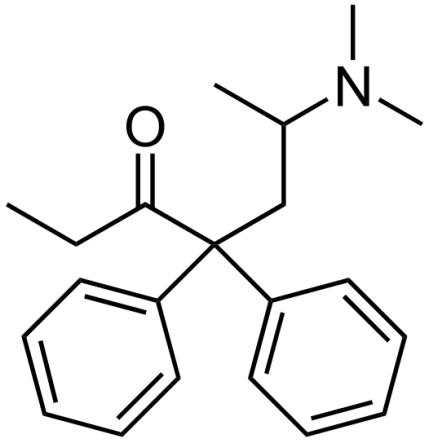
Morphine



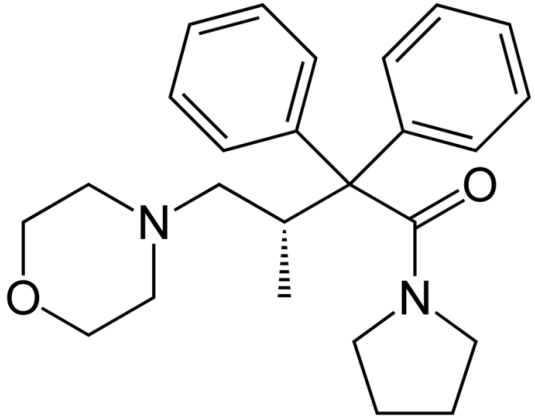
Pethidine



Ehrhardt model (1949)



Methadone



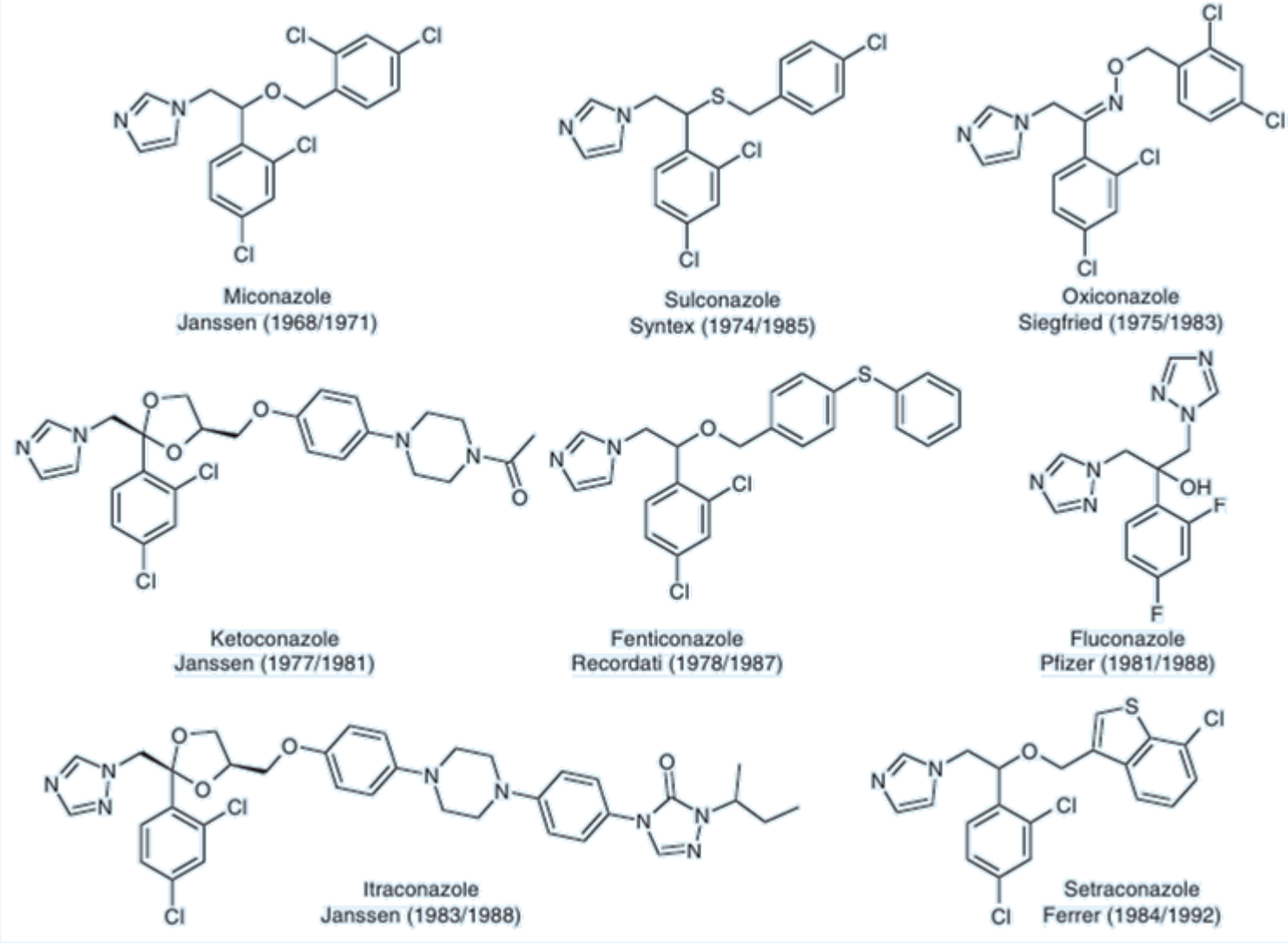
Dextromoramide

III. Rational drug design: Approaches

- Bioisosterism
- Scaffold hopping
- Twin drug approach
- Controlling of rigidity and flexibility
- Prodrugs
- etc

III. Rational drug design: Analog design

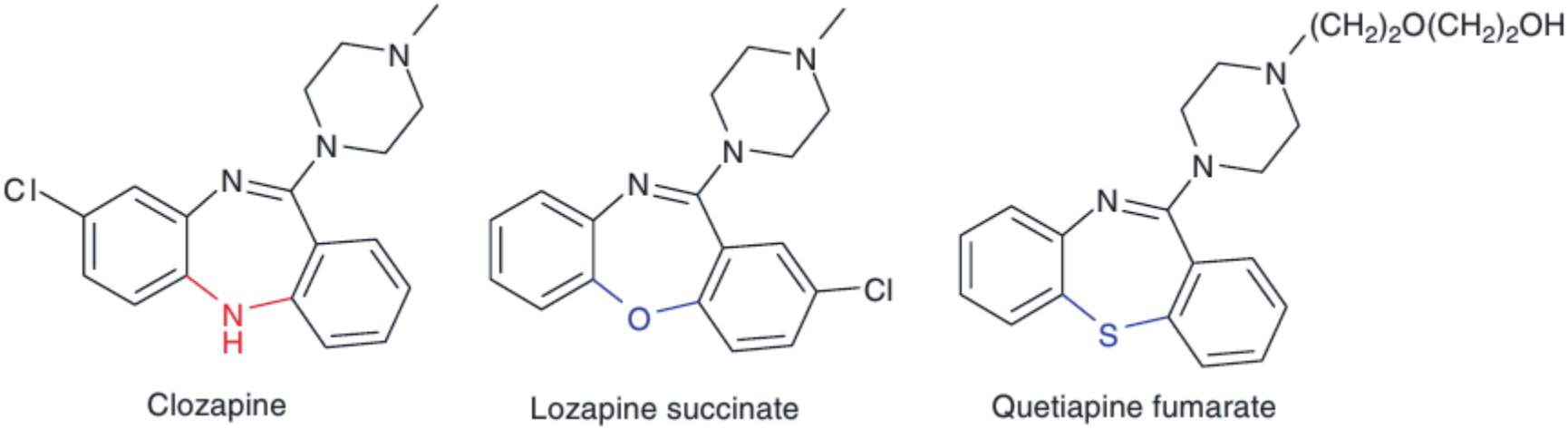
Full analogs



III. Rational drug design: Analog design

Structural analogs

an example of classical bioisosteric replacement

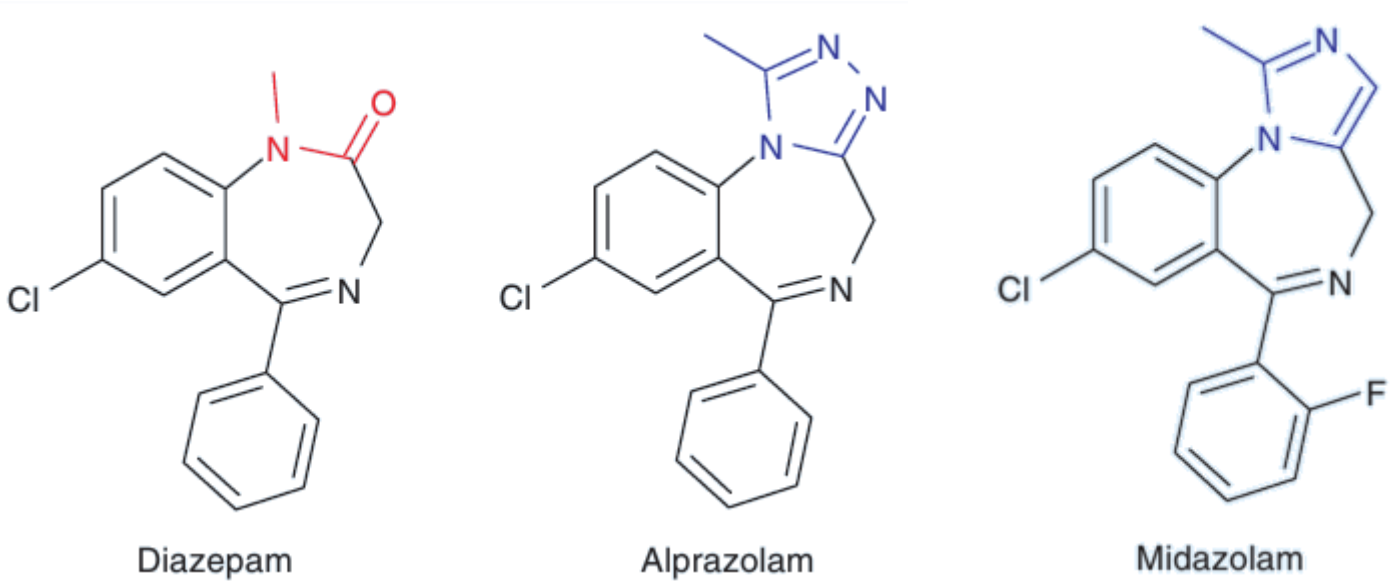


antipsychotic (schizophrenia)

III. Rational drug design: Analog design

Structural analogs

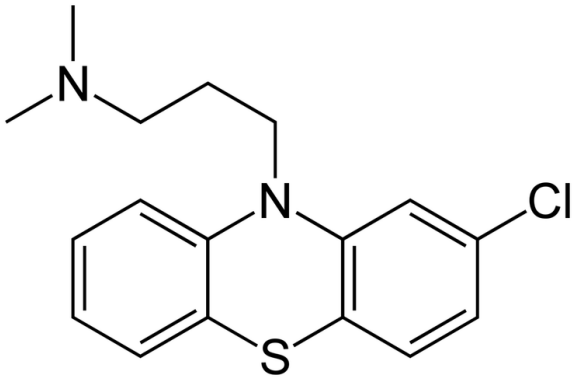
an example of non-classical bioisosteric replacement



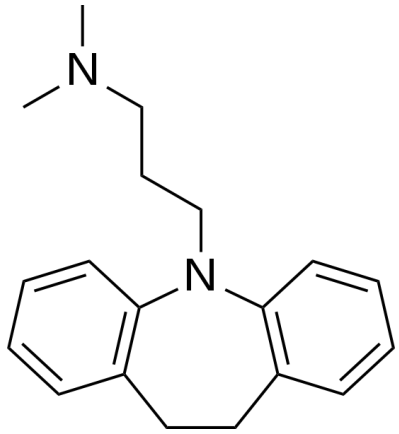
anxiolytics

III. Rational drug design: Analog design

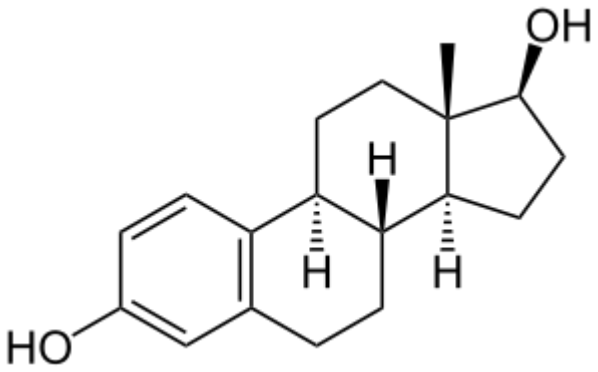
Structural analogs, which posses different activity types



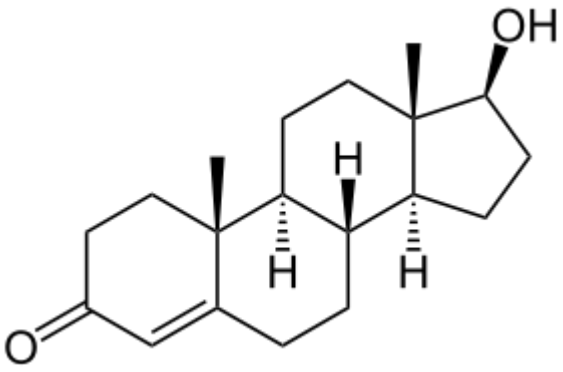
chlorpromazine
(typical antipsychotic)



Imipramine
(anti-depressant)



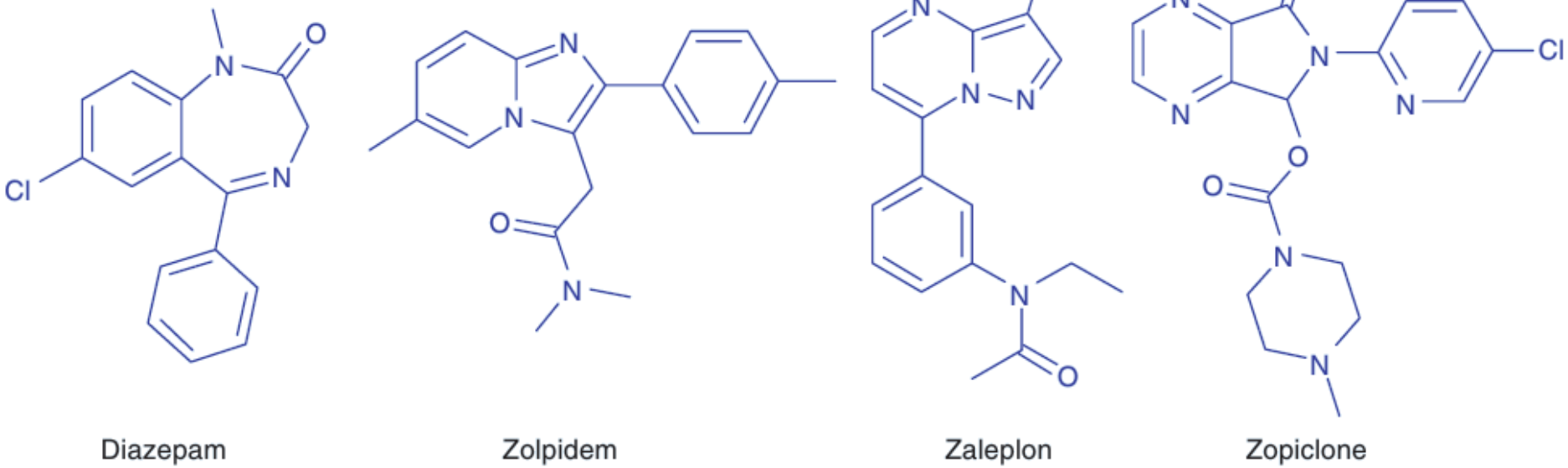
Estradiol



Testosteron

III. Rational drug design: Analog design

Functional analogs



Full agonists of GABA-A receptor

III. Rational drug design: Analog design

- Faster development
- Lower cost for fundamental research
- Known pharmacological profile and tests
- Reference compound for comparison
- Financial interest – omit licensing costs
- Totally novel activity can be found incidentally
- Low novelty and innovation levels
- Original developer of the drug has an advantage over others who are copying this drug
- Hard to conquer for the market share with the original drug which was launched earlier

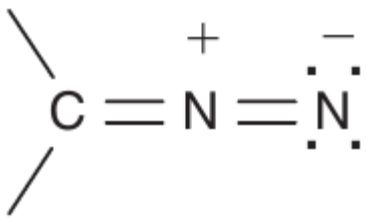
Bioisosterism: Classical isosters

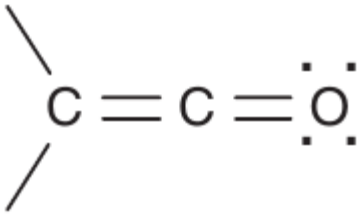
Langmuir, 1919

TABLE Groups of Isosteres as Identified by Langmuir

Groups	Isosteres
1	H^-, He, Li^+
2	O^{2-}, F^-, Ne, Na^+, Mg^{2+}, Al^{3+}
3	S^{2-}, Cl^-, Ar, K^+, Ca^{2+}
↓	↓
8	N_2, CO, CN^-
9	CH_4, NH_4^+
10	CO_2, N_2O, N^{3+}, CNO^-
↓	↓
21	SeO_4^{2-}, AsO_4^{3-}

C=O N≡N





Bioisosterism: Expansion of classical isosters

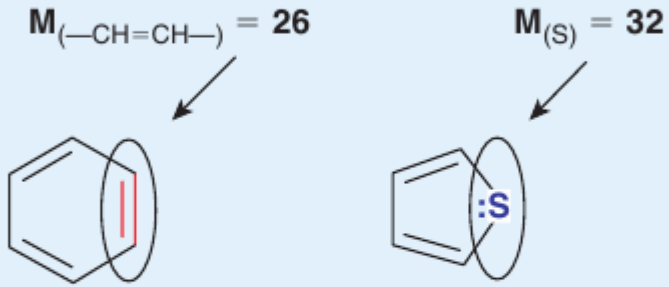
Hans Erlenmeyer , 1932

1. The whole group of element present in a given column of periodic table.

2. Pseudoatoms, which have different structures but similar properties (Cl, CN, SCN).

3. Ring equivalents (-CH=CH- and -S-)

TABLE The Sulphur Atom is Approximately Equivalent to an Ethylenic Group (Size, Mass, Capacity to provide an Aromatic Lone Pair)



Compound	E°C	Isostere	E°C
Benzene	80°	Thiophene	84°
Methylbenzene	110°	2-Methyl-thiophene	113°
Chlorobenzene	132°	2-Chloro-thiophene	130°
Acetylbenzene	200°	2-Acetyl-thiophene	214°

Bioisosterism: Definitions

Bioisosteric compounds *fit the broadest definition of isosteres and have the same type of biological activity*.

Friedman H.L. Infuence of isosteric replacements upon biological activity. NASNRS 1951, 206, 295–358.

Bioisosteres are groups or molecules which have chemical and physical similarities producing broadly similar biological effects

Thornber C.W. Isosterism and molecular modification in drug design. Chem. Soc. Rev. 1957, 8, 563–580.

Bioisosterism: Burger's classification

TABLE Classic Bioisostere Atoms and Groups

Monovalent	Divalent	Trivalent	Tetravalent
—OH, —NH_2, —CH_3, —OR	—CH_2—	=CH—	=C=
—F, —Cl, —Br, —I, —SH, —PH_2	—O—	=N—	=Si=
—Si_3, —SR	—S—	=P—	=N^+=
	—Se—	=As—	=P^+=
	—Te—	=Sb—	=As^+=
			=Sb^+=

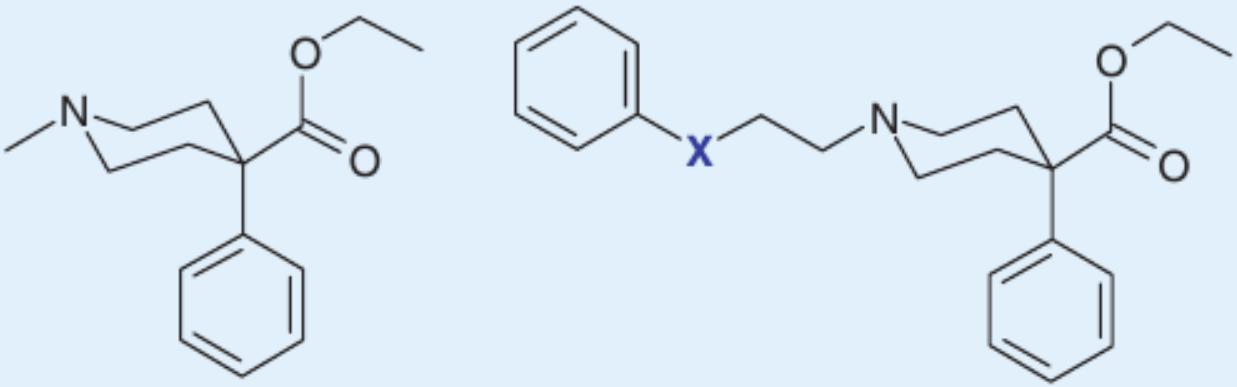
Bioisosterism: Burger's classification

TABLE Non-Classical Isosteres

—CO—	—COOH	—SO_2NH_2	—H	—CONH—	—COOR	—$CONH_2$
—CO_2—	—SO_3H	—$PO(OH)NH_2$	—F	—NHCO—	—ROCO—	—$CSNH_2$
—SO_2—	—tetrazole					
—SO_2NR—	—SO_2NHR —SO_2NH_2		—OH —CH_2OH		—catechol	
—CON—	—3-hydroxyisoxazole				—benzimidazole	
—CH(CN)—	—2-hydroxychromones		—$NHCONH_2$			C_4H_4S
R—S—R			—NH—CS—NH_2			—C_5H_4N
(R—O—R)	=N—					—C_6H_5
R—N(CN)—	C(CN)=R′		—NH—C(=$CHNO_2$)—NH_2 —NH—C(=CHCN)—NH_2			
—halide						—C_4H_4NH
	—CF_3					
	—CN					
	—$N(CN)_2$					
	—$C(CN)_3$					

Bioisosterism: replacement of divalent atoms

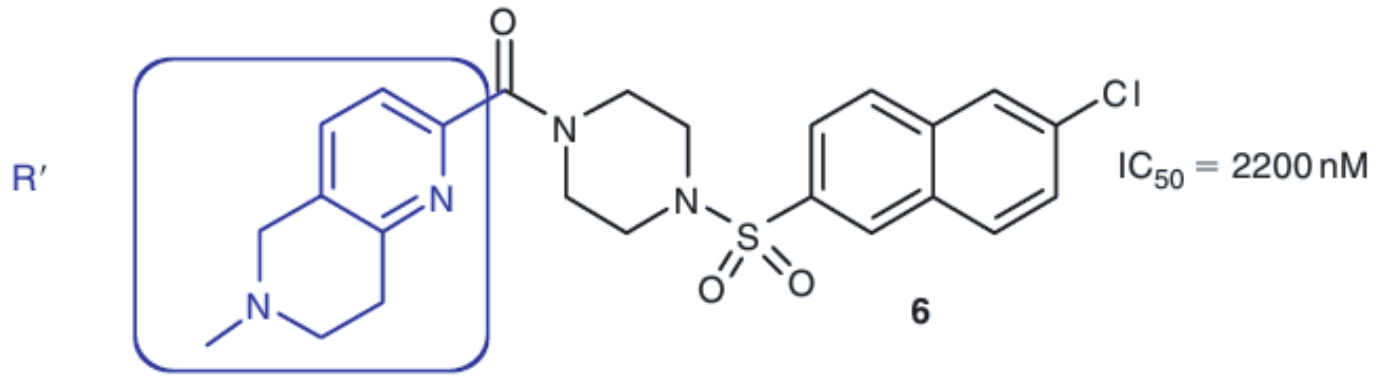
TABLE Meperidine Analogs



Meperidine

X	Analgesic potency (Meperidine = 1)
O	12
NH	80
CH_2	20
S	1,5

Bioisosterism: ring replacement



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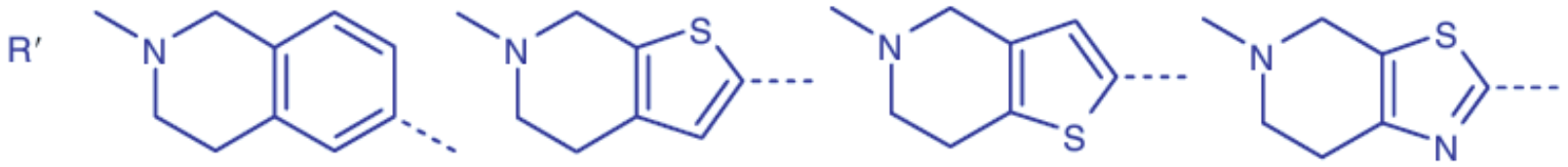
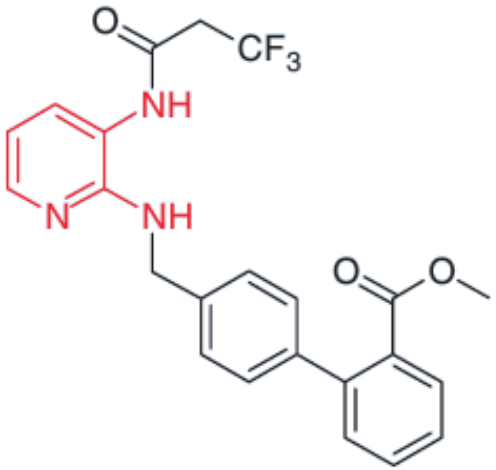
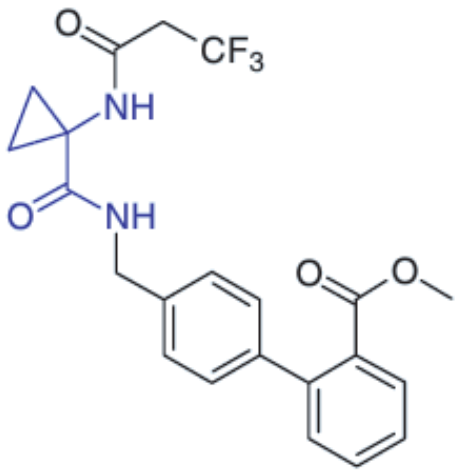


Anti factor Xa activities IC_{50} (nM)	1200	105	190	22

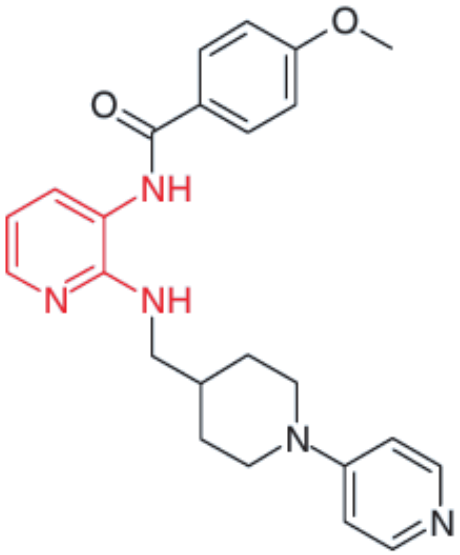
Bioisosterism: ring replacement



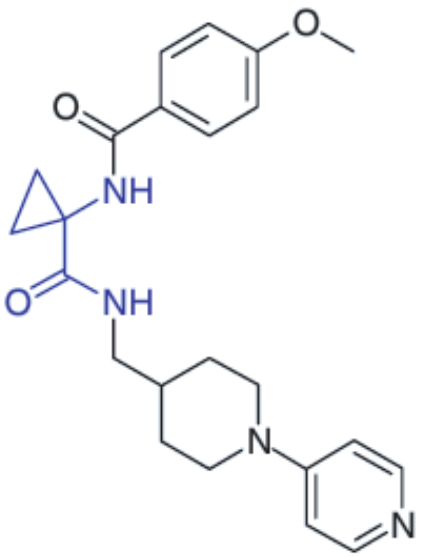
7, Human BK B_1 K_1 = 11.8 nM



8, Human BK B_1 K_1 = 63.0 nM

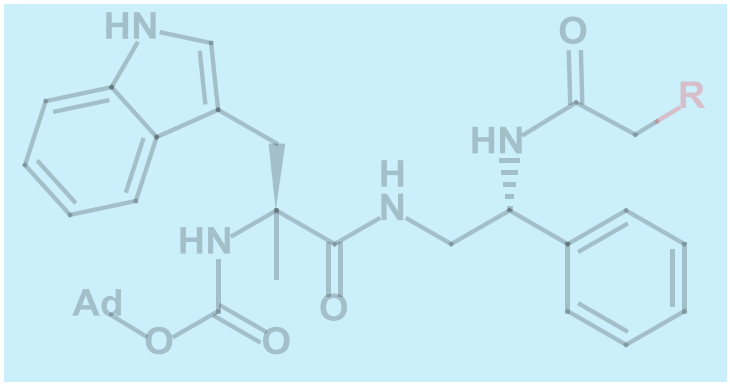


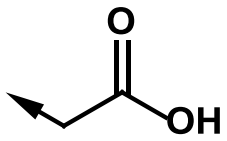
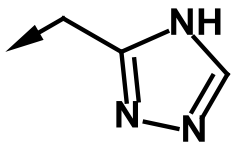
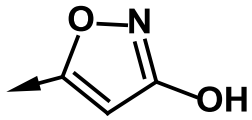
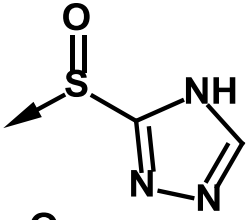
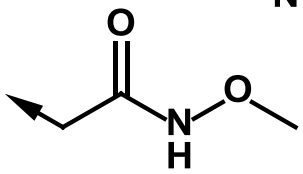
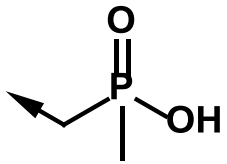
9, Human BK B1 Ki > 10 μM
Human factor Xa Ki = 39 nM



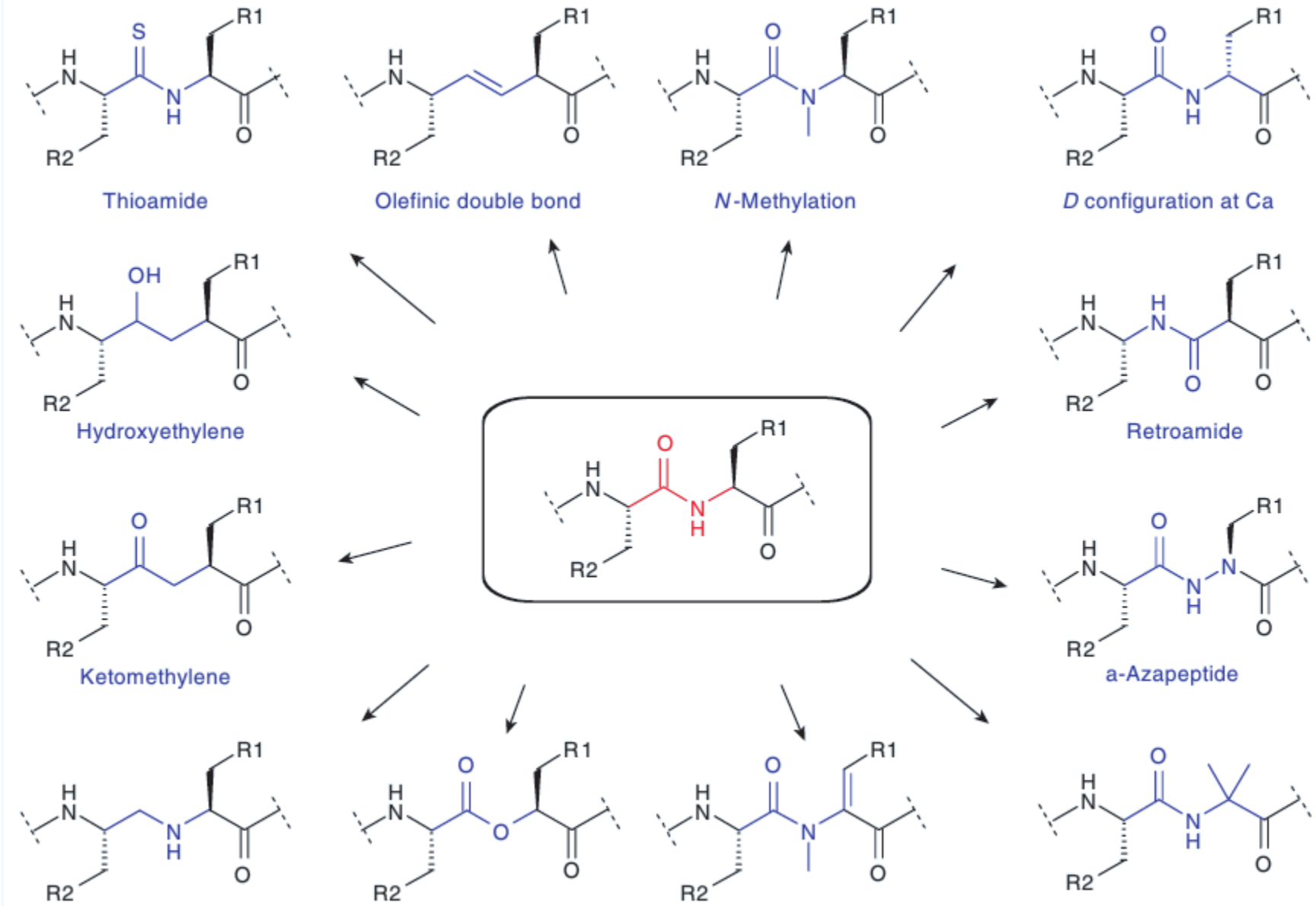
10, Human BK B_1 Ki > 10 μM
Human factor Xa Ki = 175 nM

Bioisosterism: Bioisosters of carboxylic acid group

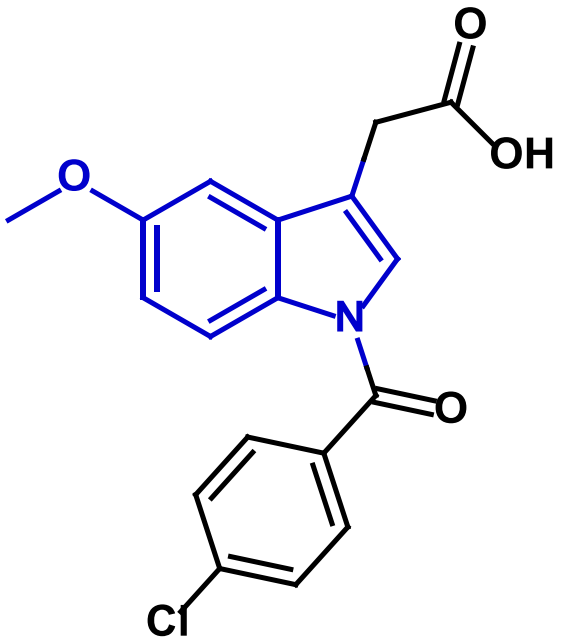


	CCK-A IC_{50} (nM)	CCK-B IC_{50} (nM)	A/B	pK_a
	1.7	4500	2500	5.6
	6.0	970	160	5.4
	2.6	1700	650	6.5
	1.7	940	550	7.0
	21	1500	71	>9.5
	23	4400	190	3.7

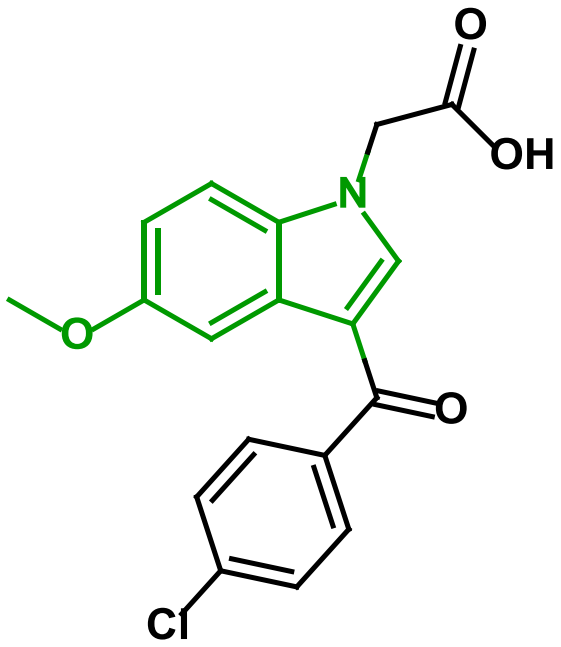
Bioisosterism: amide bond bioisosters



Scaffold hopping



Indometacin

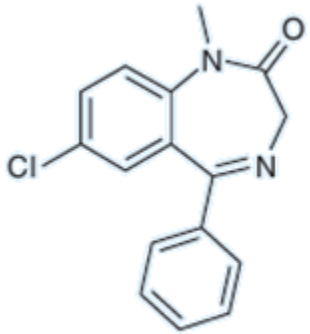


Clometacine

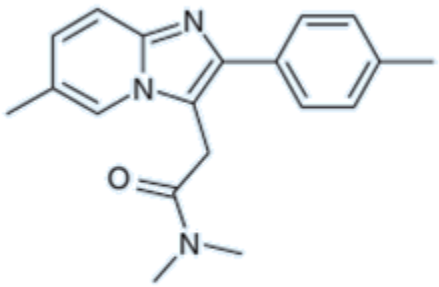
(anti-inflammatory drugs)

Scaffold hopping

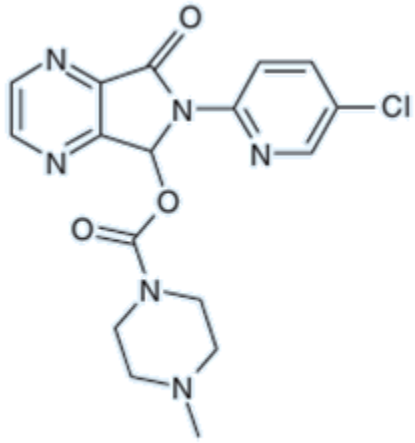
Full GABA-A agonists



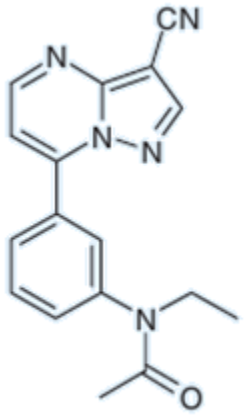
Diazepam



Zolpidem



Zopiclone

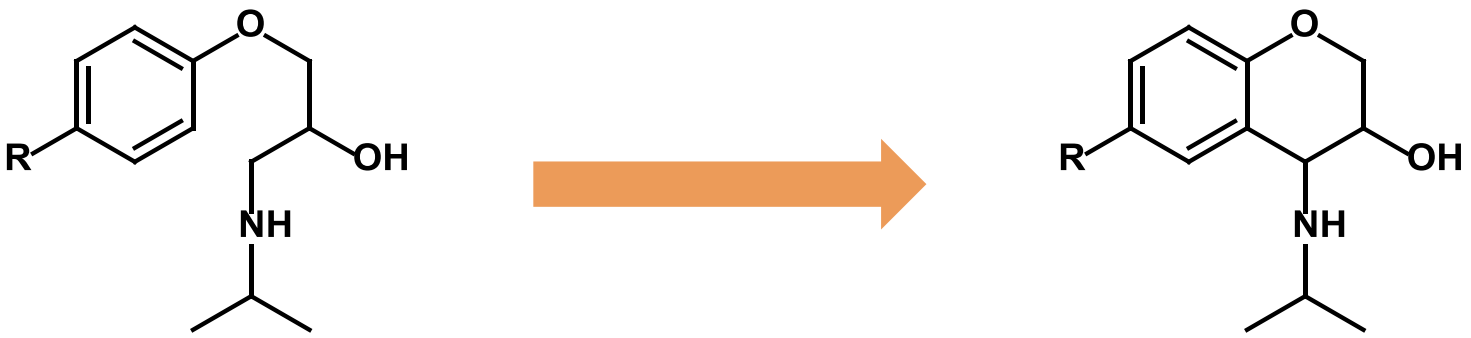


Zaleplon

Controlling rigidity/flexibility

Steric factors

pre-ordering of interaction sites of ligand



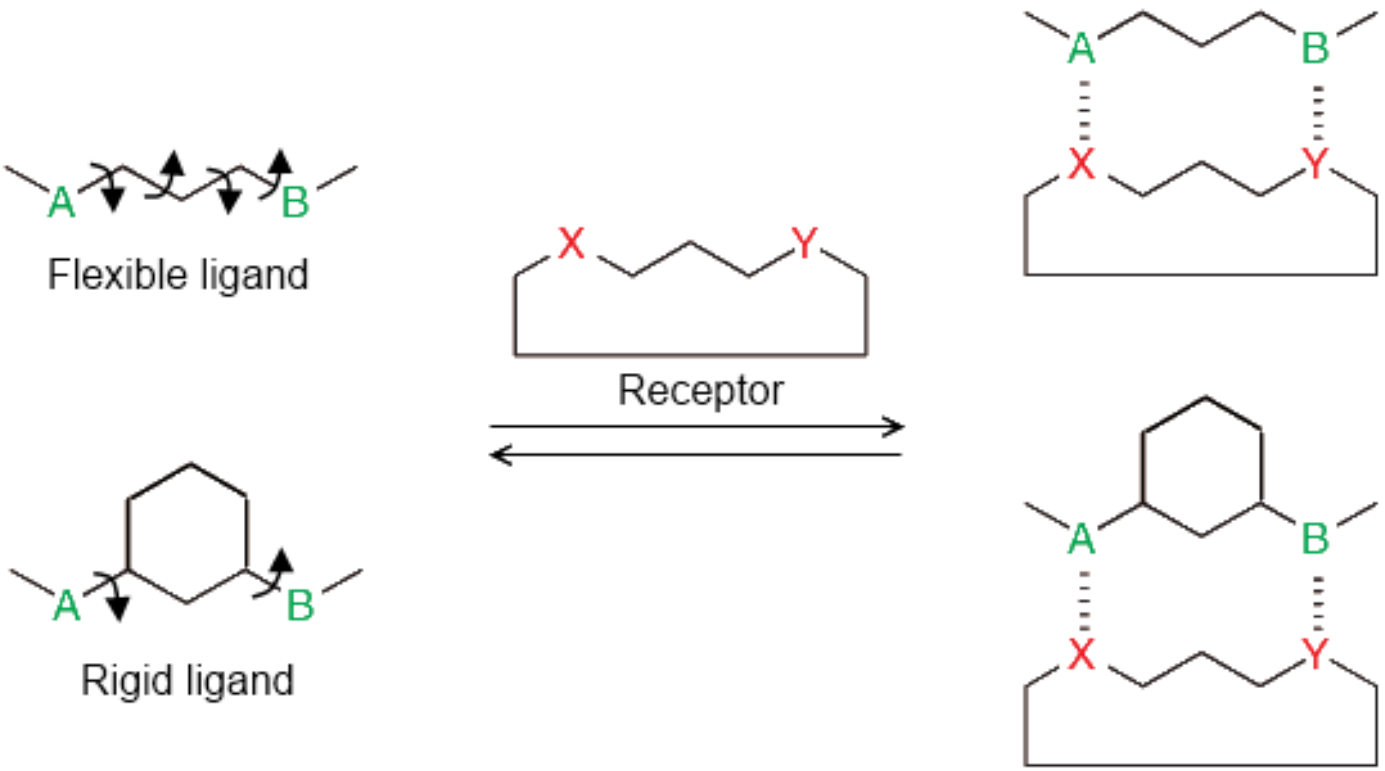
Propanolol
β-adrenergic receptor blocker
(anxiety, panic, hypertension)

anti-hypertensive activity
no β-blocking activity

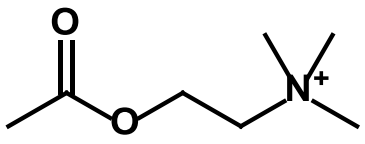
Controlling rigidity/flexibility

Balance between enthalpy and entropy terms

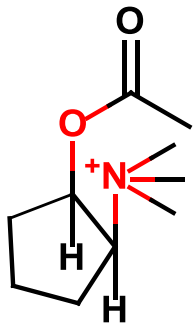
entropy-enthalpy compensation

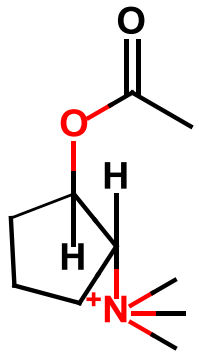


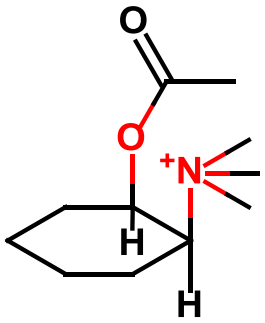
Controlling rigidity/flexibility

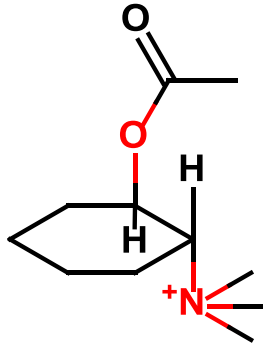


Acetylcholine









	C5-cis	C5-trans	C6-cis	C6-trans
N..O distance, (Å)	2.51	3.45	2.5-2.9	2.9-3.7
Relative cholinergic activity	1.43	1.07	1.14	1.06

Controlling rigidity/flexibility

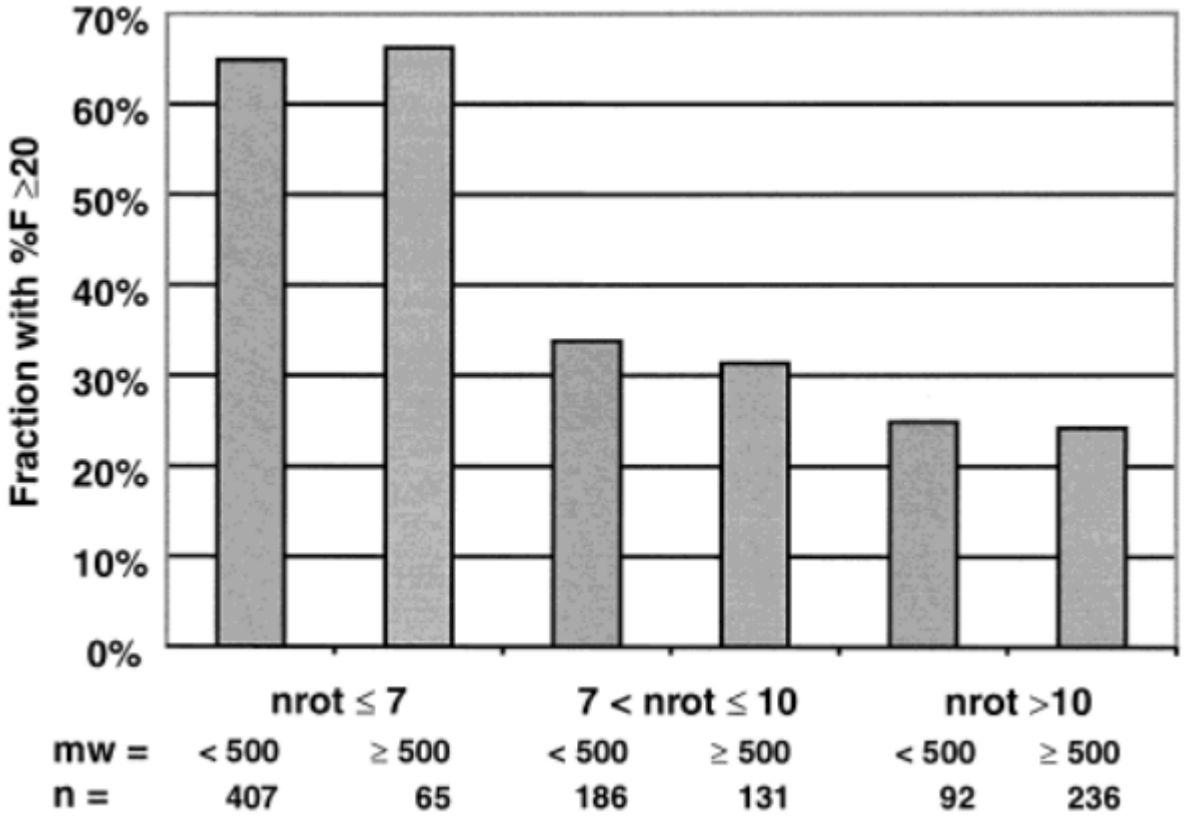
	Cell adhesion to laminin fragment P1, IC_{50} (µM)		
	A375	HBL-100	HT1080
cyclo(Arg-Gly-Asp-Phe-D-Val)	1.9	0.9	1.0
Arg-Gly-Asp-Phe-D-Val	29	42	92

Aumailley M. et al, *FEBS Letters*, 7 October 1991, 291, 1, p.50-54.

Controlling rigidity/flexibility

Bioavailability

the lower number of rotatable bonds the higher chance of high bioavailability



Veber D.F. et al, *J. Med. Chem.*, **2002**, *45* (12), pp 2615–2623

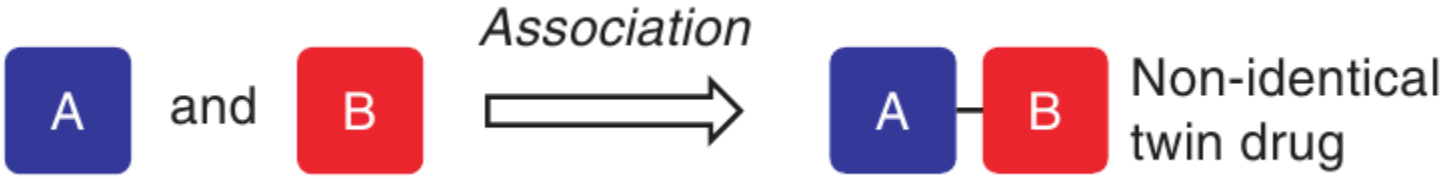
Twin drug approach

Homodimers

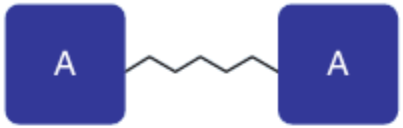
higher activity and/or selectivity

Heterodimers

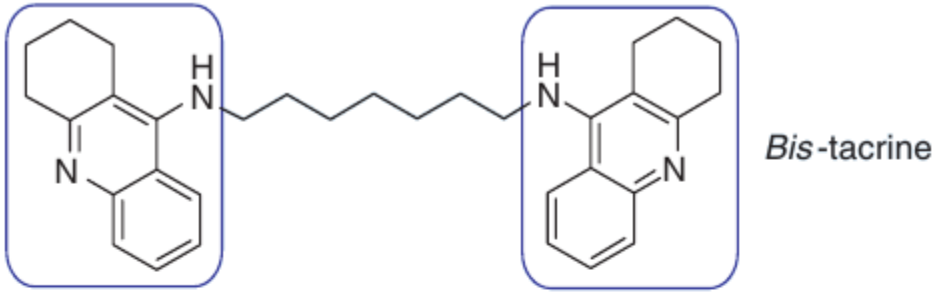
synergistic effect of simultaneous modulation of two targets



Twin drug approach



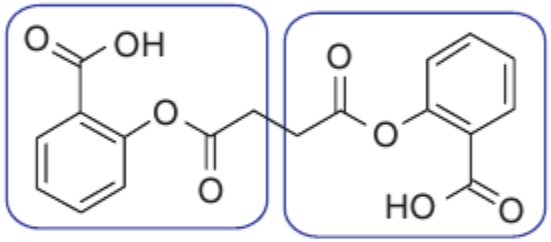
Linker mode



Bis-tacrine

Tacrine Tacrine

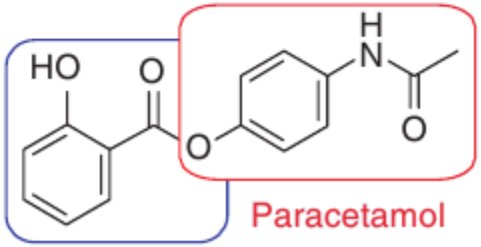
No linker mode



Diaspirin

Acetylsalicylic acid Acetylsalicylic acid

Overlap mode



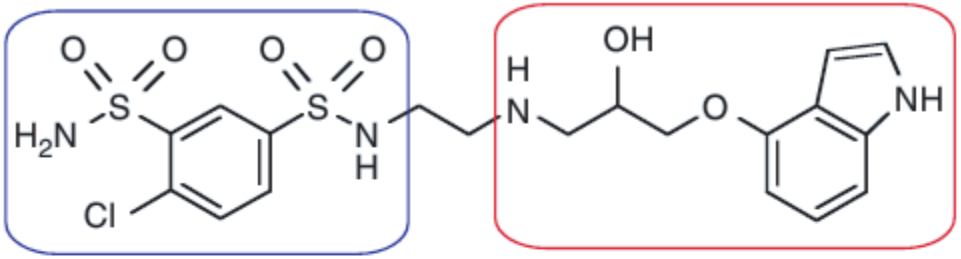
Acetaminosalol

Paracetamol

Salicylic acid

Twin drug approach: Degree of overlapping of twin drugs

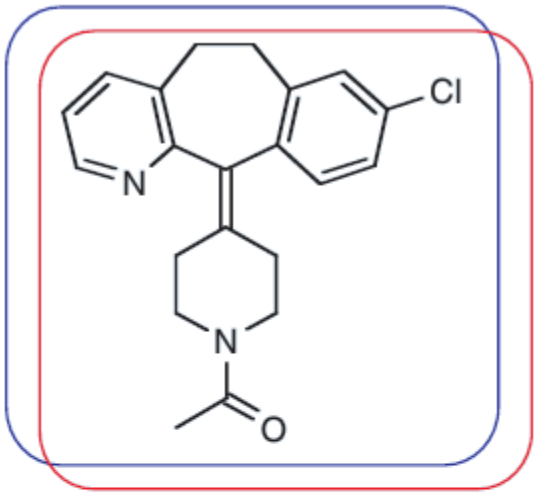
Associative synthesis



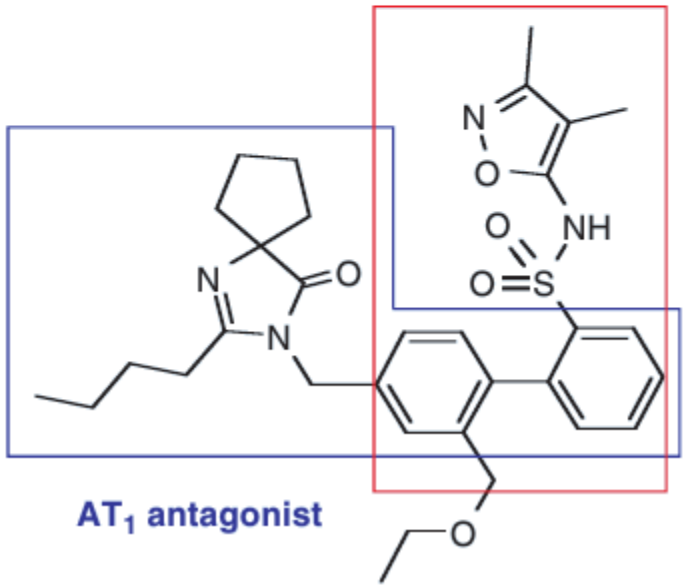
Diuretic agent

β Adrenergic antagonist (β blocker)

Intrinsically dual acting drug



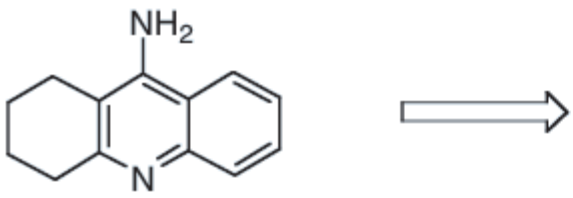
PAF antagonist
H_1 antagonist



ET_A antagonist

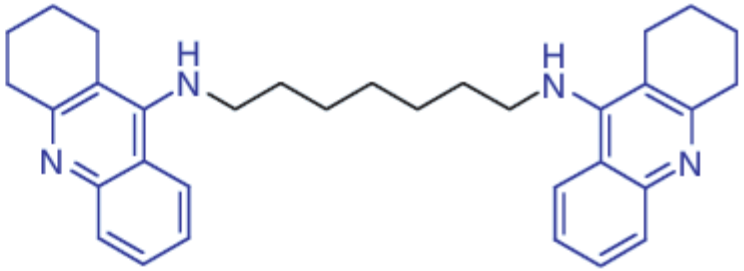
AT_1 antagonist

Twin drug approach: Selectivity and potency changes



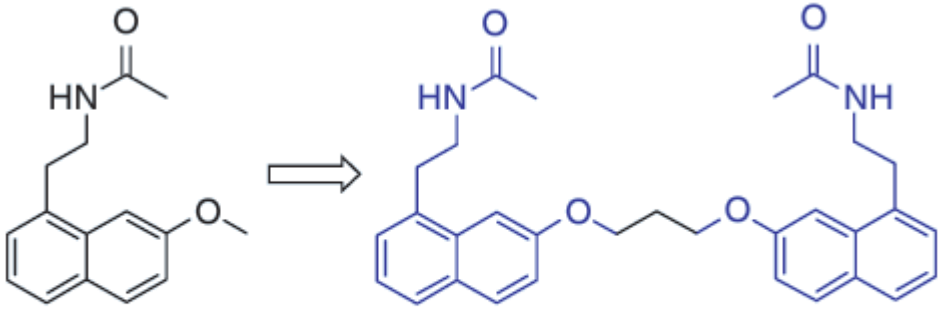
Tacrine

AChE: IC_{50} = 333 nM
AChE selectivity: 0.3



Bis (7)-tacrine

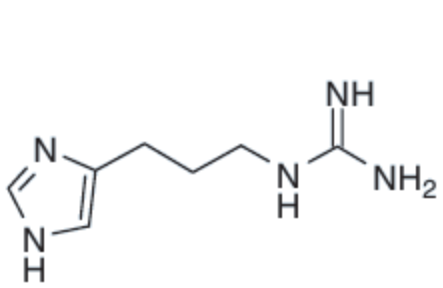
AChE: IC_{50} = 0.2 nM
AChE selectivity: 221



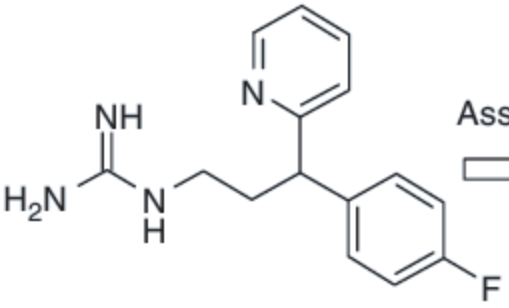
Agomelatine
MT_1: K_i = 0.06 nM
MT_2 / MT_1: 4

Bis-agomelatine
MT_1: K_i = 0.50 nM
MT_2 / MT_1: 224

Twin drug approach: Selectivity and potency changes

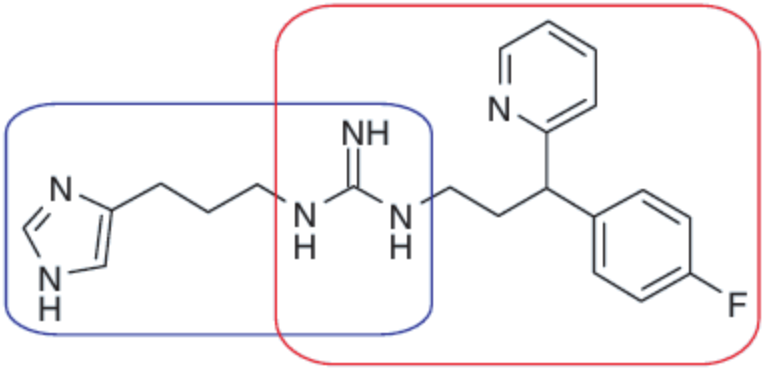


Weak H_2 agonist



Weak H_1 antagonist

Association



Arpromidine

H_1 antagonist: $pA_2 = 7.65$
H_2 agonist: $pD_2 = 8.0$

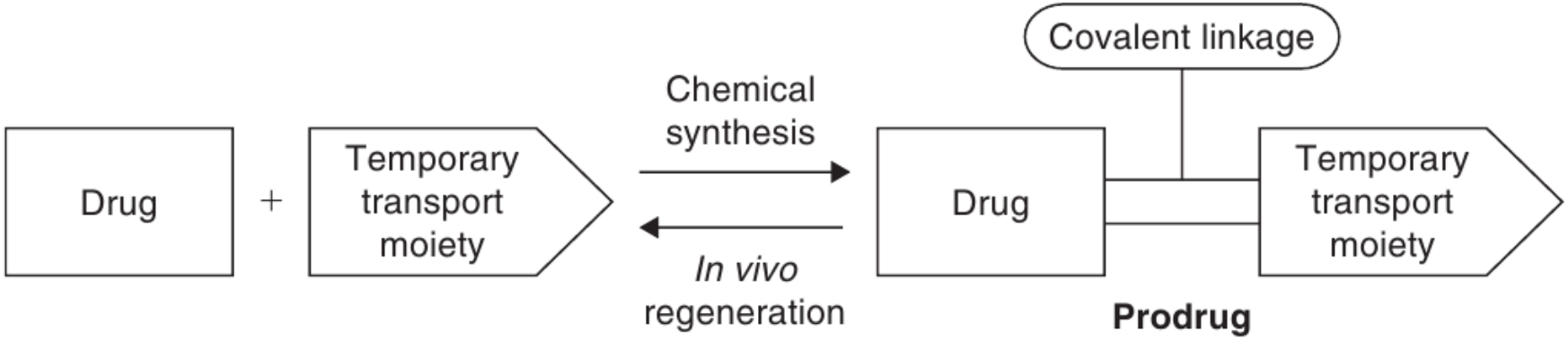
Prodrugs

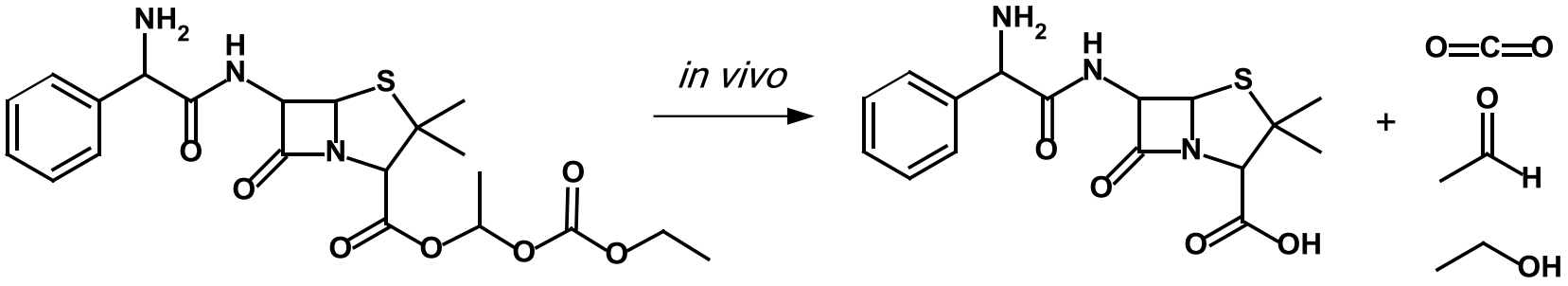
Prodrug design is “the chemical modification of a biologically active compound to form a new compound that, upon *in vivo* enzymatic attack, will liberate the parent compound”

Harper N.J., *J. Med. Pharm. Chem.* 1959, 1, 467–500.

Prodrugs: classification

Carrier prodrugs

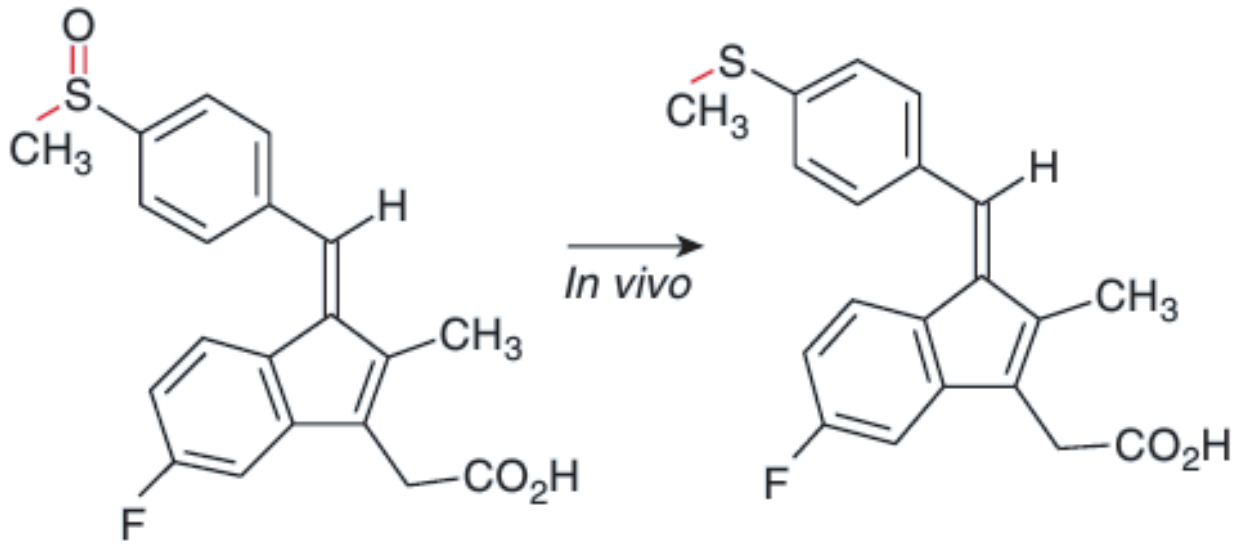




bacampicillin

ampicillin

Bioprecursors

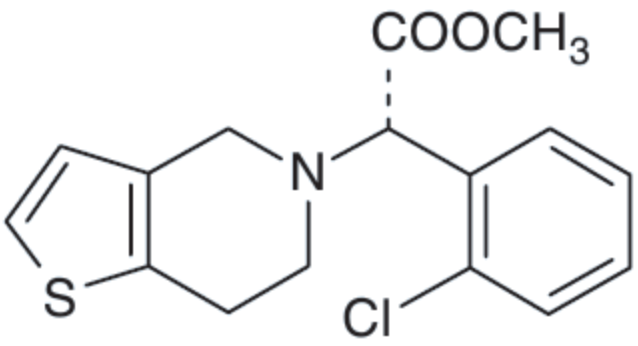


Sulindac

inactive *in vitro*

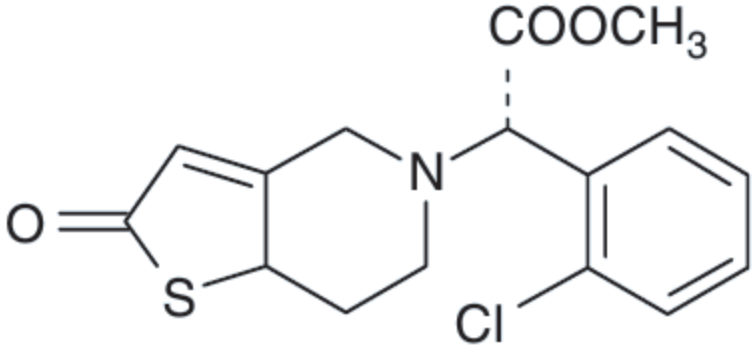
active *in vitro*

Bioprecursors: An example



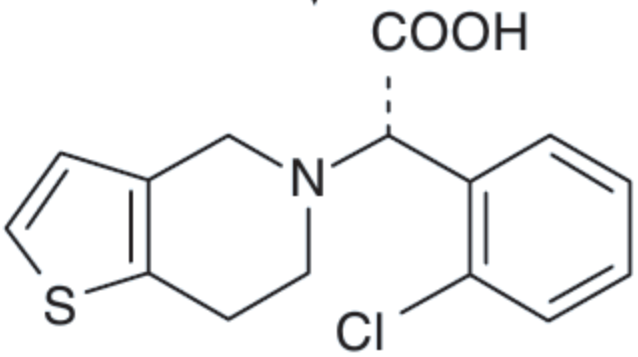
Clopidogrel

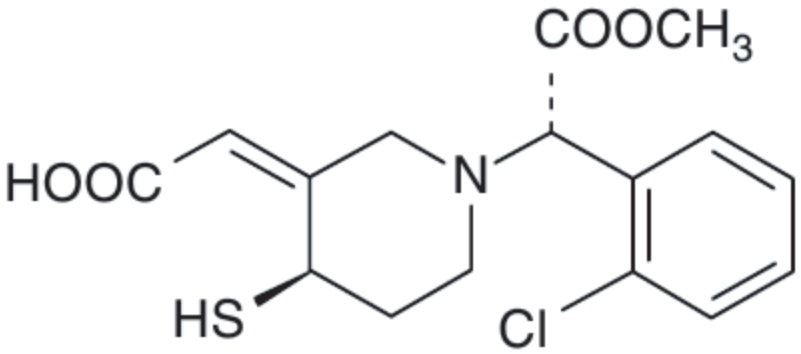
P450 3A
activation



2-Oxo-clopidogrel

Hydrolysis





Savi P. et al, *Thromb. Haemostasis* 2000, 84, 891–896

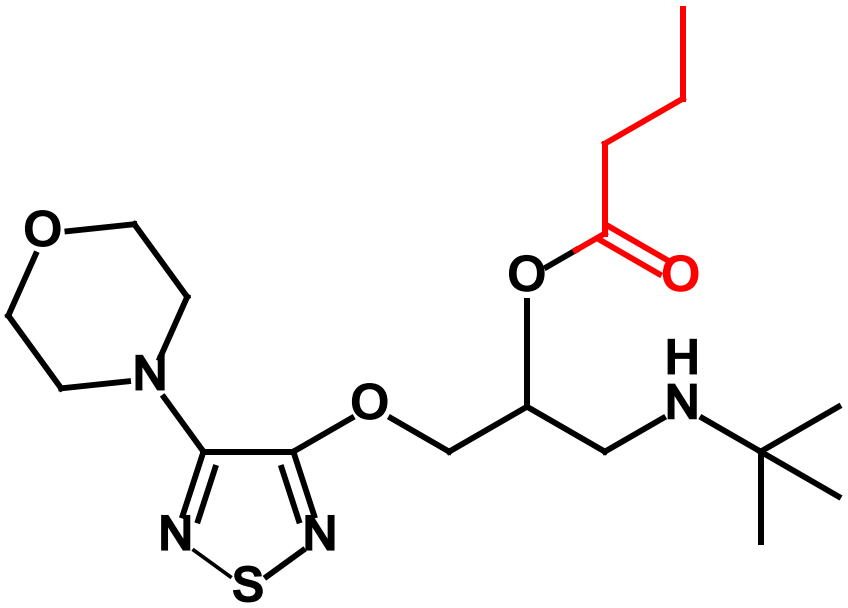
Carrier prodrugs vs. Bioprecursors

	Prodrugs	
	Carrirer prodrugs	Bioprecursors
Constitution	Active principle + carrier group	No carrier group
Lipophilicity	Strongly modified	Usually slightly modified
Bioactivation	Hydrolytic	Mostly oxidative or reductive
Catalysis	Chemical or enzymatic	Only enzymatic

Prodrugs: Applications

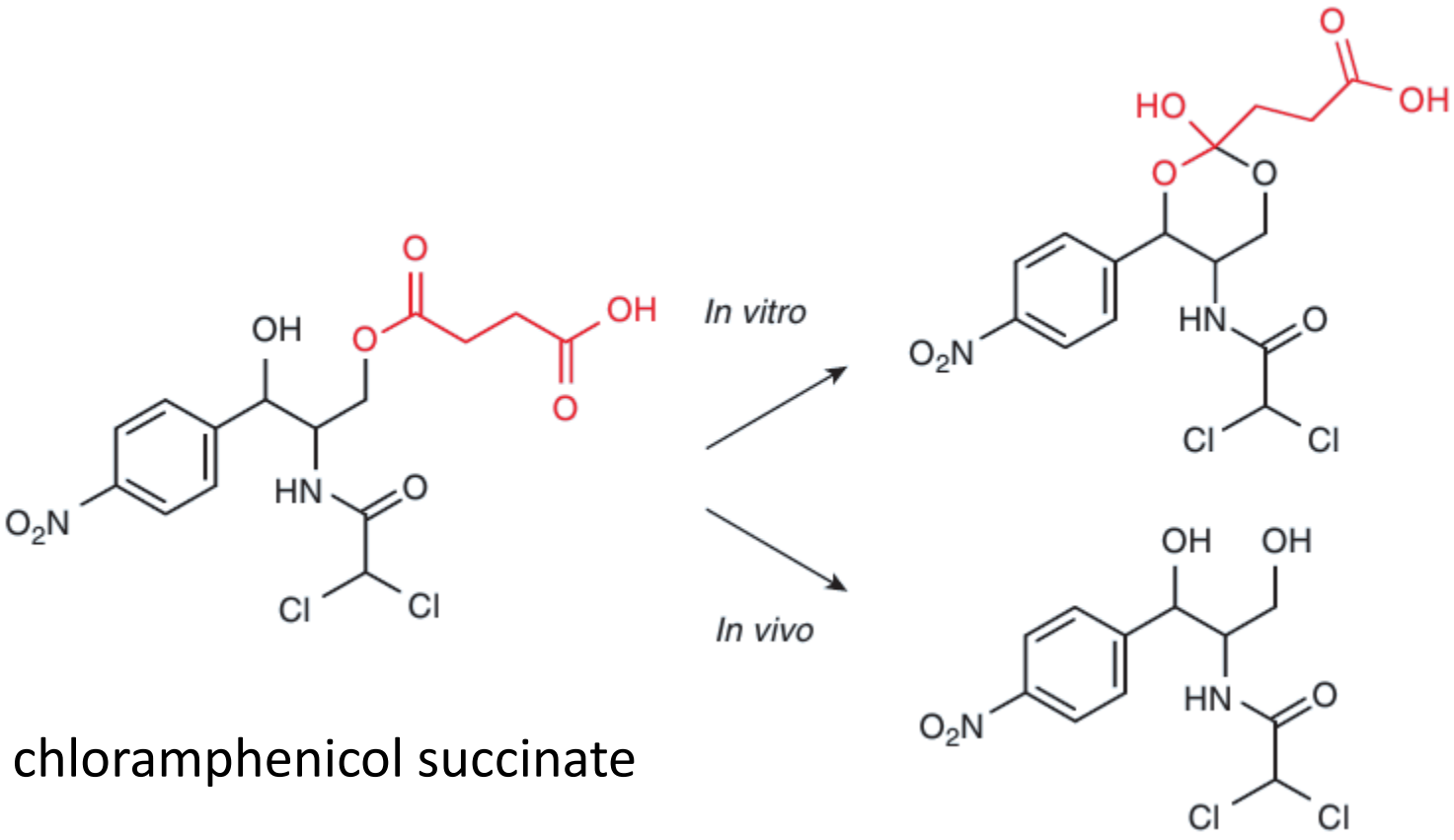
1. Increase lipophilicity
2. Increase duration of pharmacological effect
3. Increase site-specificity
4. Decrease toxicity and adverse effects
5. Improvement in drug formulation
   stability, water solubility, suppression of an undesirable organoleptic or physicochemical properties

Prodrugs applications: increasing of lipophilicity



Timolol	logP = -0.04 (pH=7.4)
Butyryl-timolol	logP = 2.08 (pH=7.4)

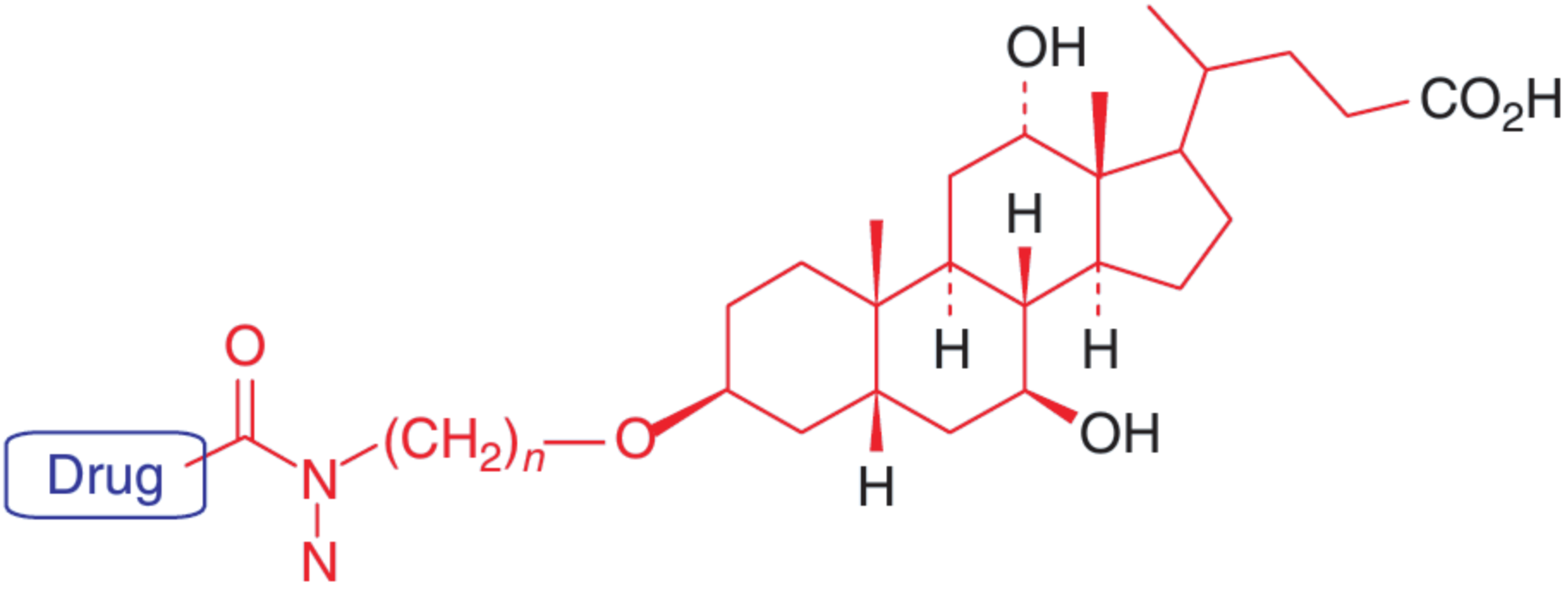
Prodrugs applications: improvement of drug formulation



chloramphenicol succinate

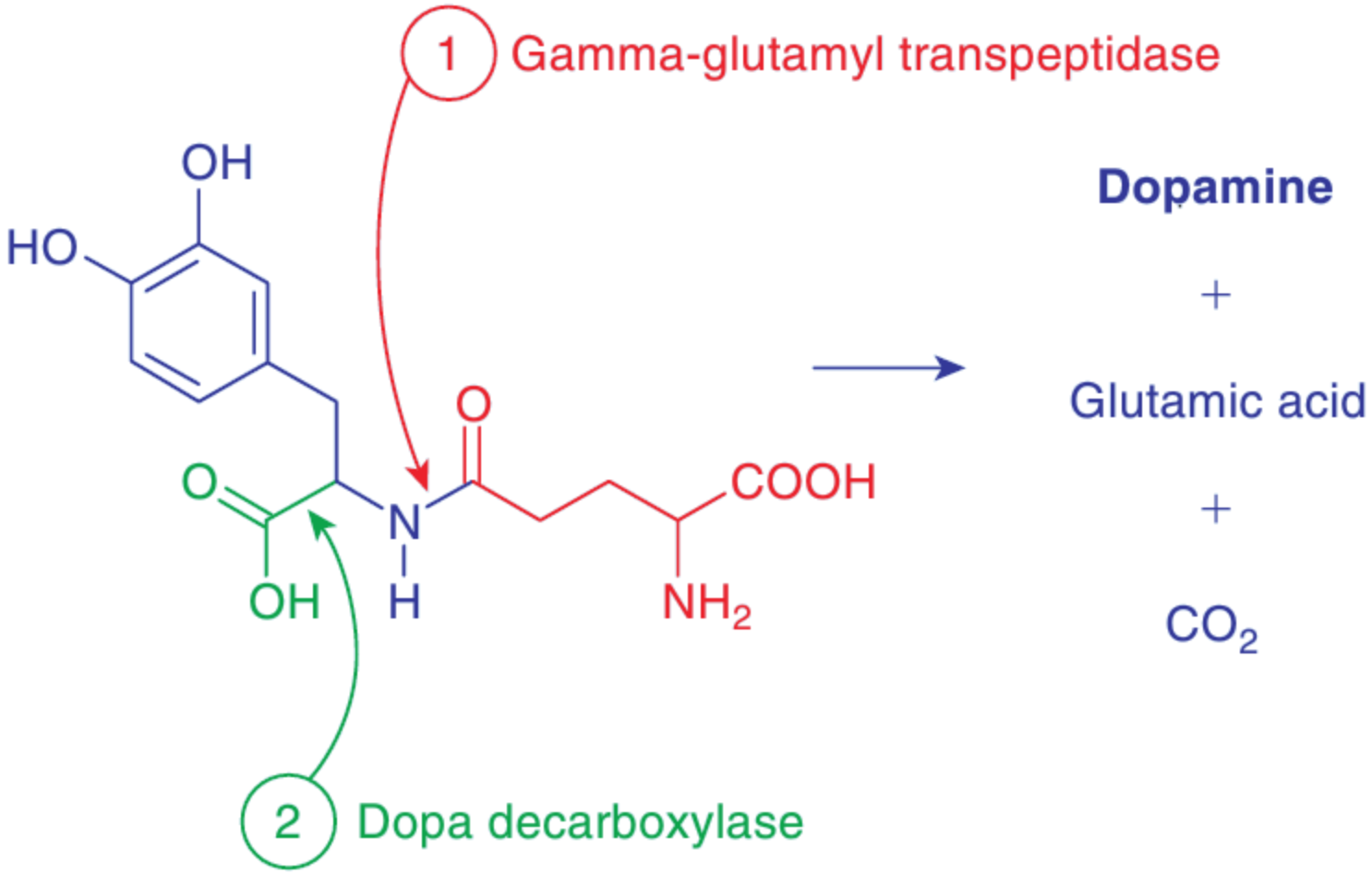
Prodrugs applications: site-specific delivery

Liver-specific targeting



endogenous bile acid transport system

Prodrugs applications: site-specific release



γ-glutamyldopa

selective renal vasodilator

Prodrugs: Limitations and difficulties

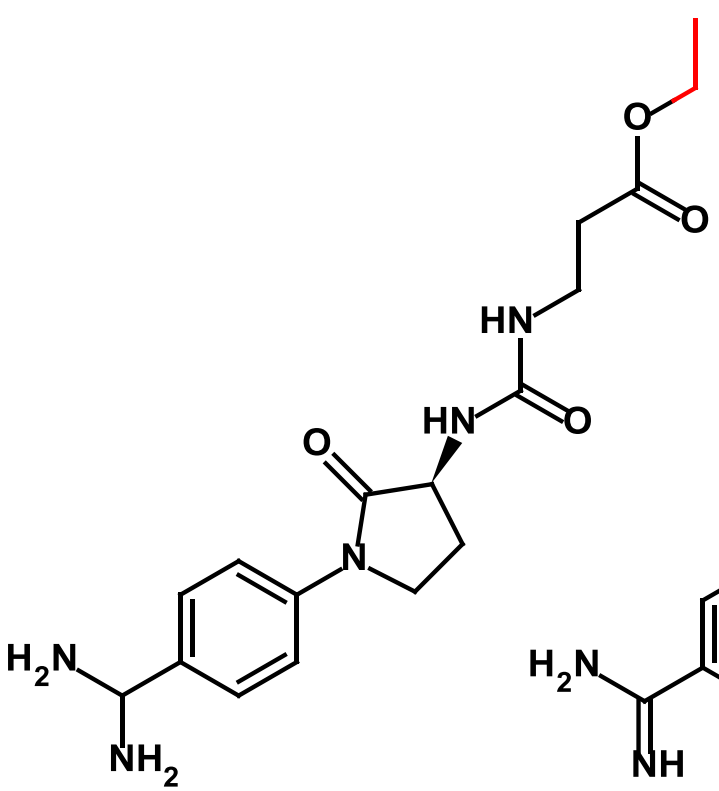
1. Imposibility of in vitro testing
   because bioactivation is necessary to create the active species

2. Possible misinterpretation of pharmacokinetic profiles
   pivampicillin: $t_{1/2}$ in buffered aqueous solution at 37°C
   103 min, in the presence of 10% human serum
   50 min, in the whole human blood 5 min.

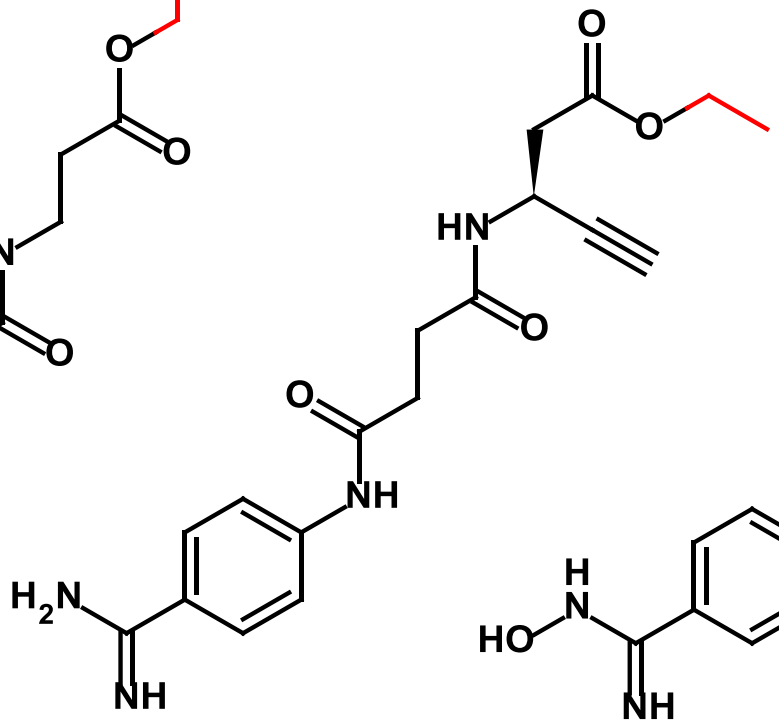
3. Possible toxicological
   toxicity of the whole prodrug, unexpected metabolites, consumption of vital constituent during prodrug activation, etc

Prodrugs: Limitations and difficulties

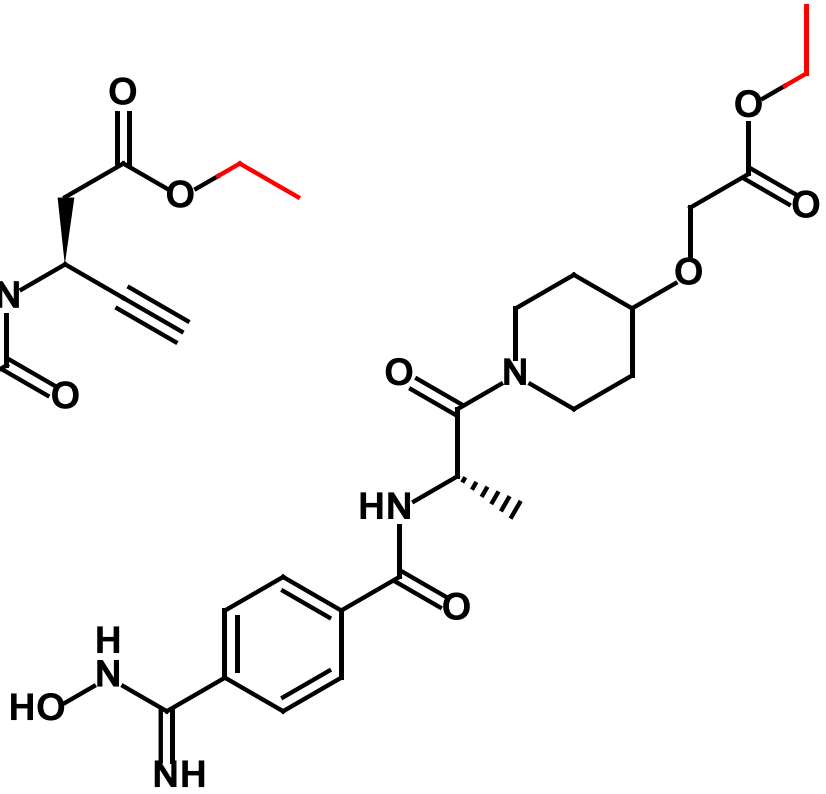
Antagonists of fibrinogen receptor (anti-platelet therapy)



Orbofiban



Xemilofiban



Sibrafiban

Chew D.P. et al, *Circulation*, **2001,** 103, p.201-206.

Computer-aided drug design (CADD)

	Unknown protein structure	**Known protein structure**
Unknown ligands structures	CADD of no use	***De novo* design**
Known ligands structures	**Ligand-based design** QSAR, pharmacophore modeling, similarity searching	**Structure-based design** Molecular docking, pharmacophore modeling

Recommended literature

The practice of medicinal chemistry. Third edition. Edited by C.G. Wermuth.