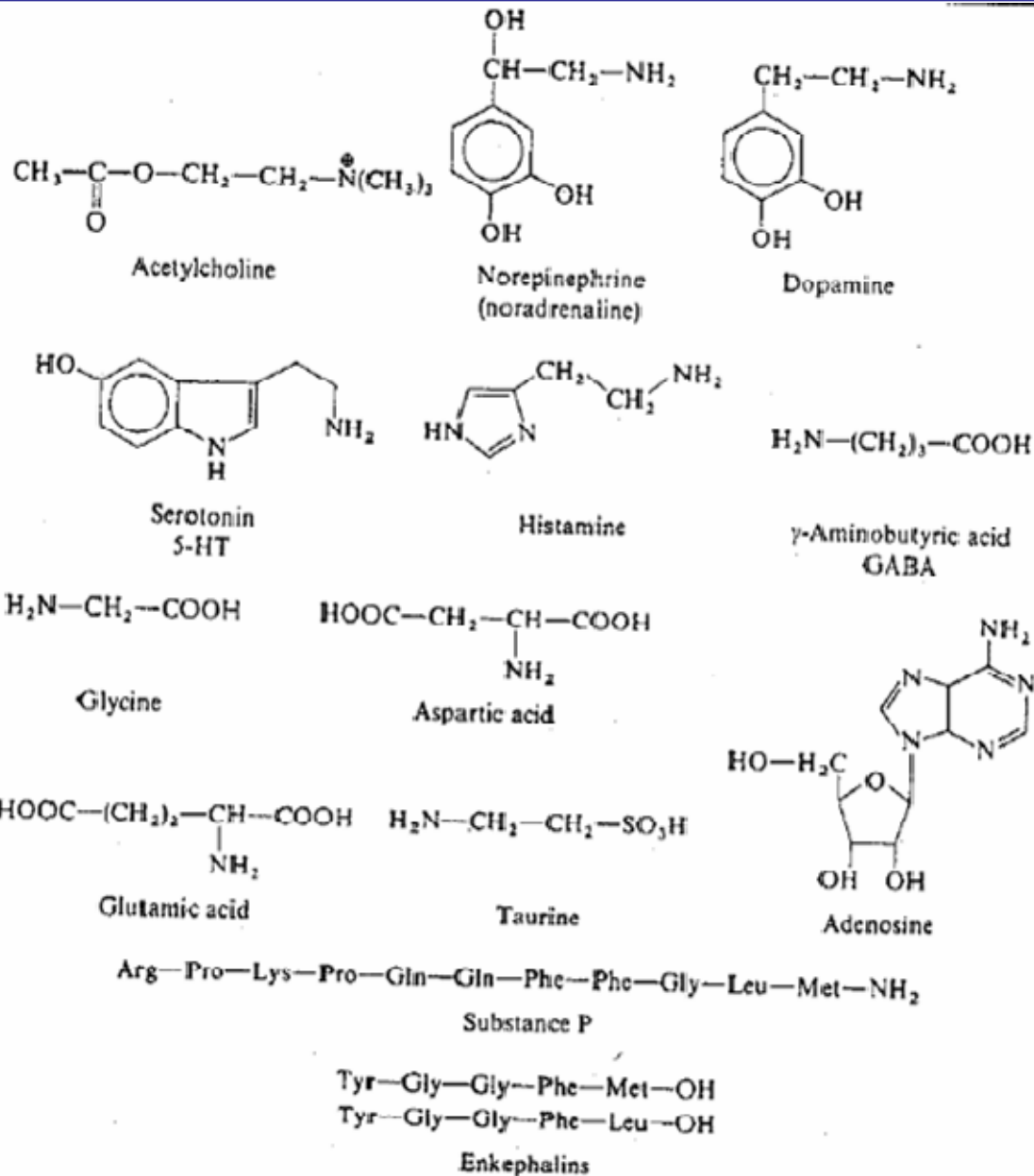# HIGHLIGHTS IN DRUG DESIGN

BOURGUIGNON Jean - Jacques (CNRS)
Laboratoire de Pharmacochimie Moléculaire
Faculté de Pharmacie
74, route du Rhin, BP 24
67401 ILLKIRCH, France.

jjb@pharma.u-strasbg.fr

Web: http://bourguignonlab.u-strasbg.fr

Structure of some neurotransmitters

Fig.1 : Aminoacid sequences and model for the organization of the dopamine D_2 and D_3 receptors, two members of the large superfamily of receptors coupled to G proteins.
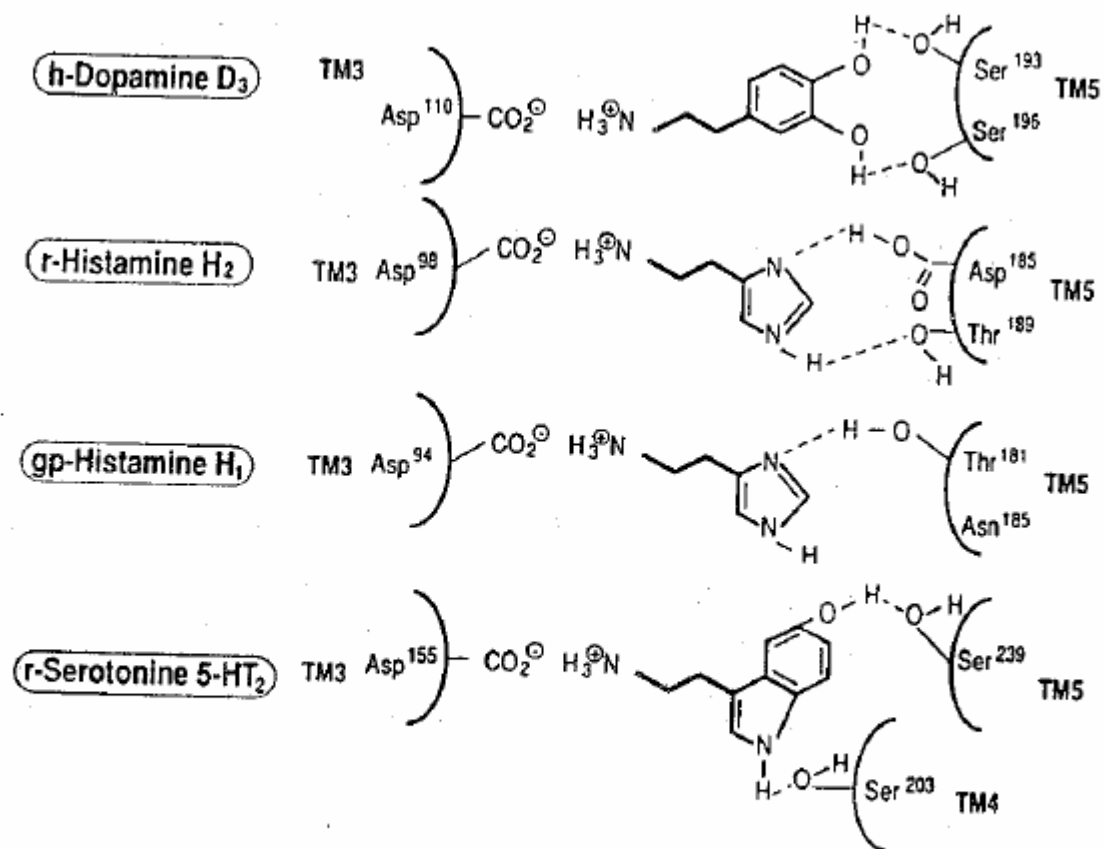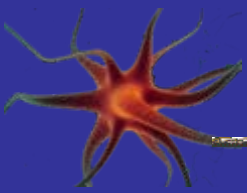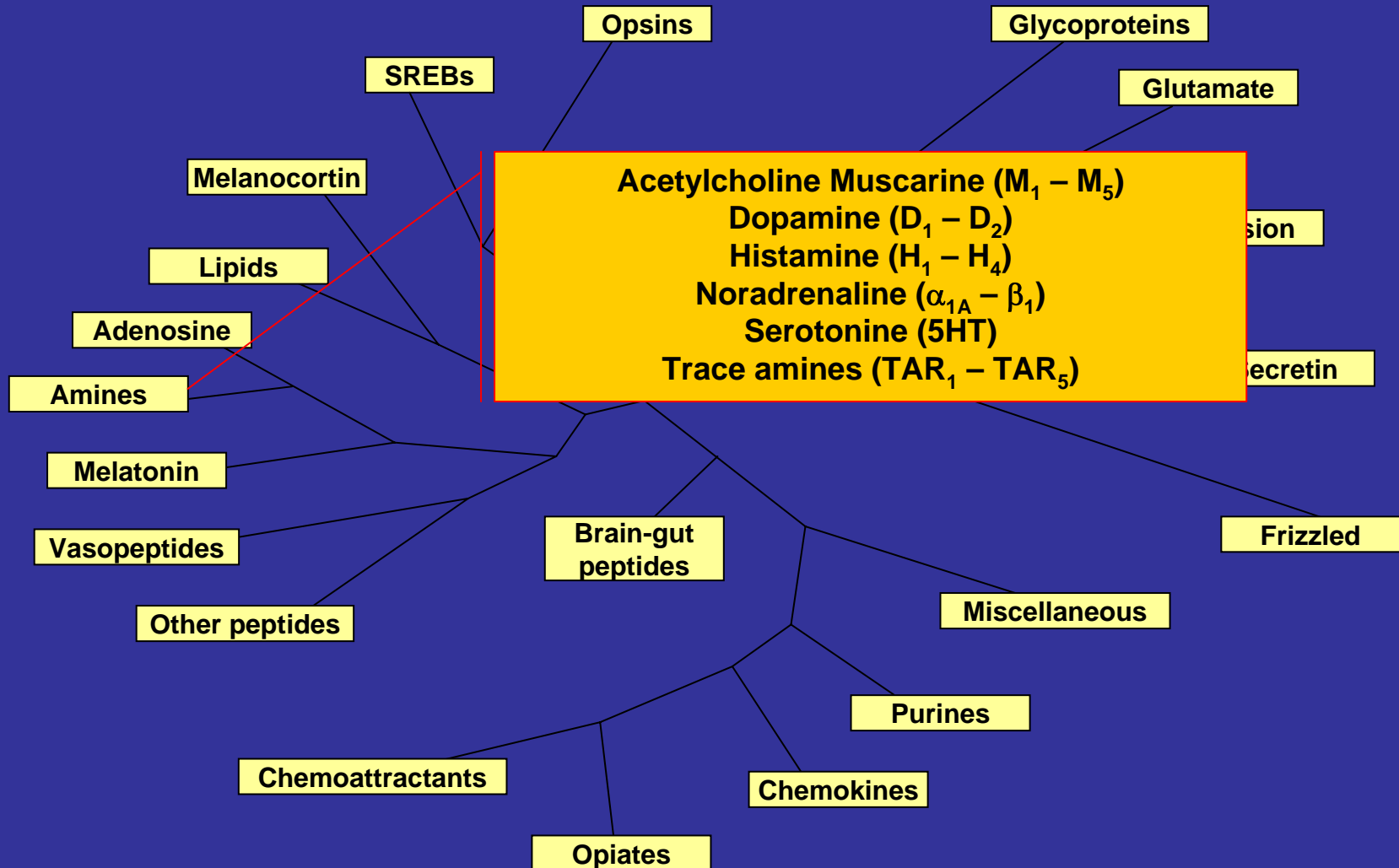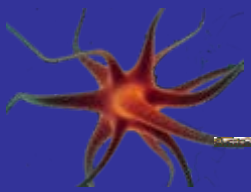
Fig.2 : Putative modes of interaction of some amines with aminoacid residues in transmembrane domains (TMs) of their respective receptors.
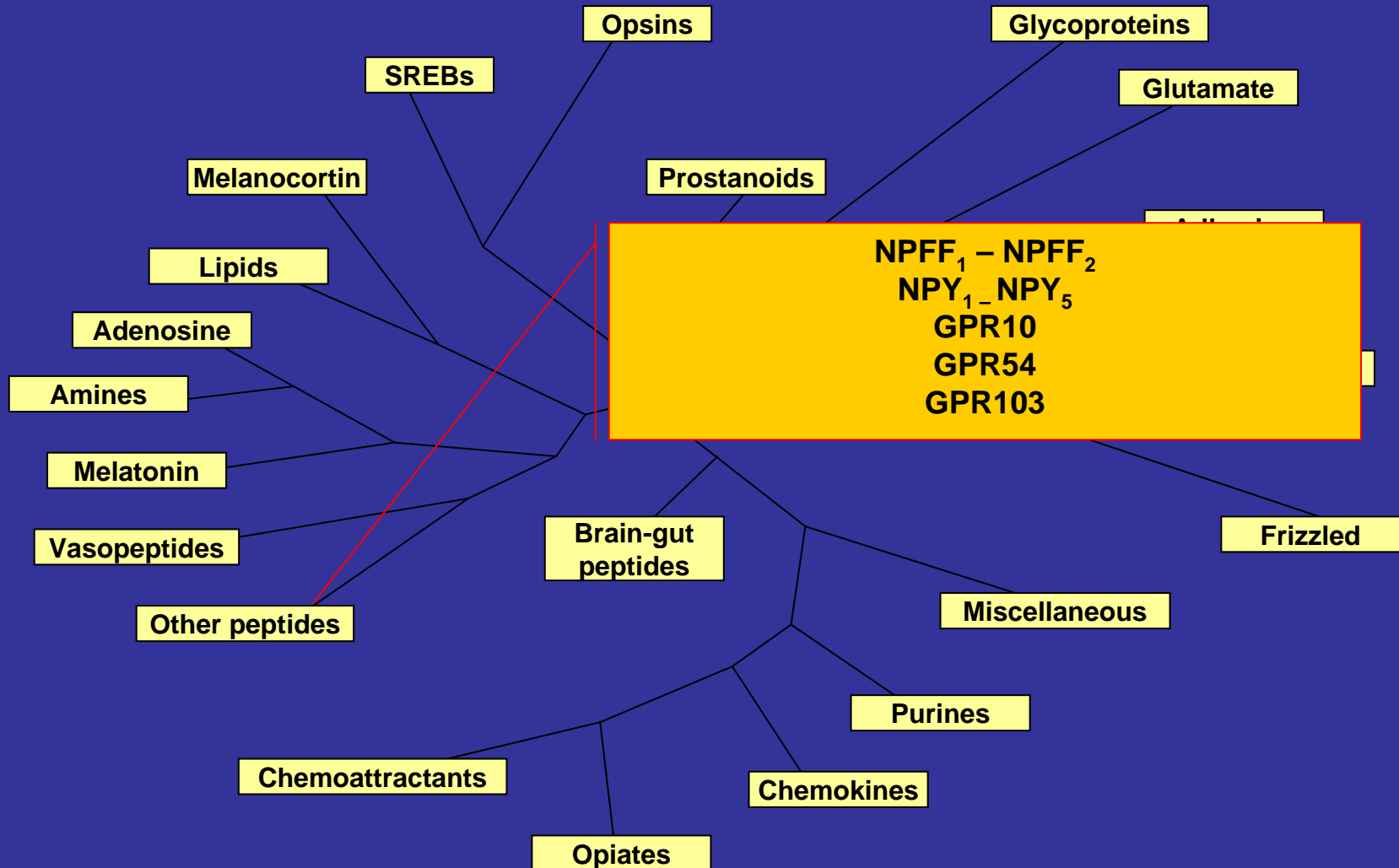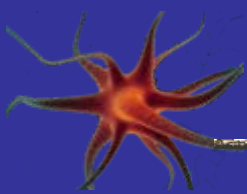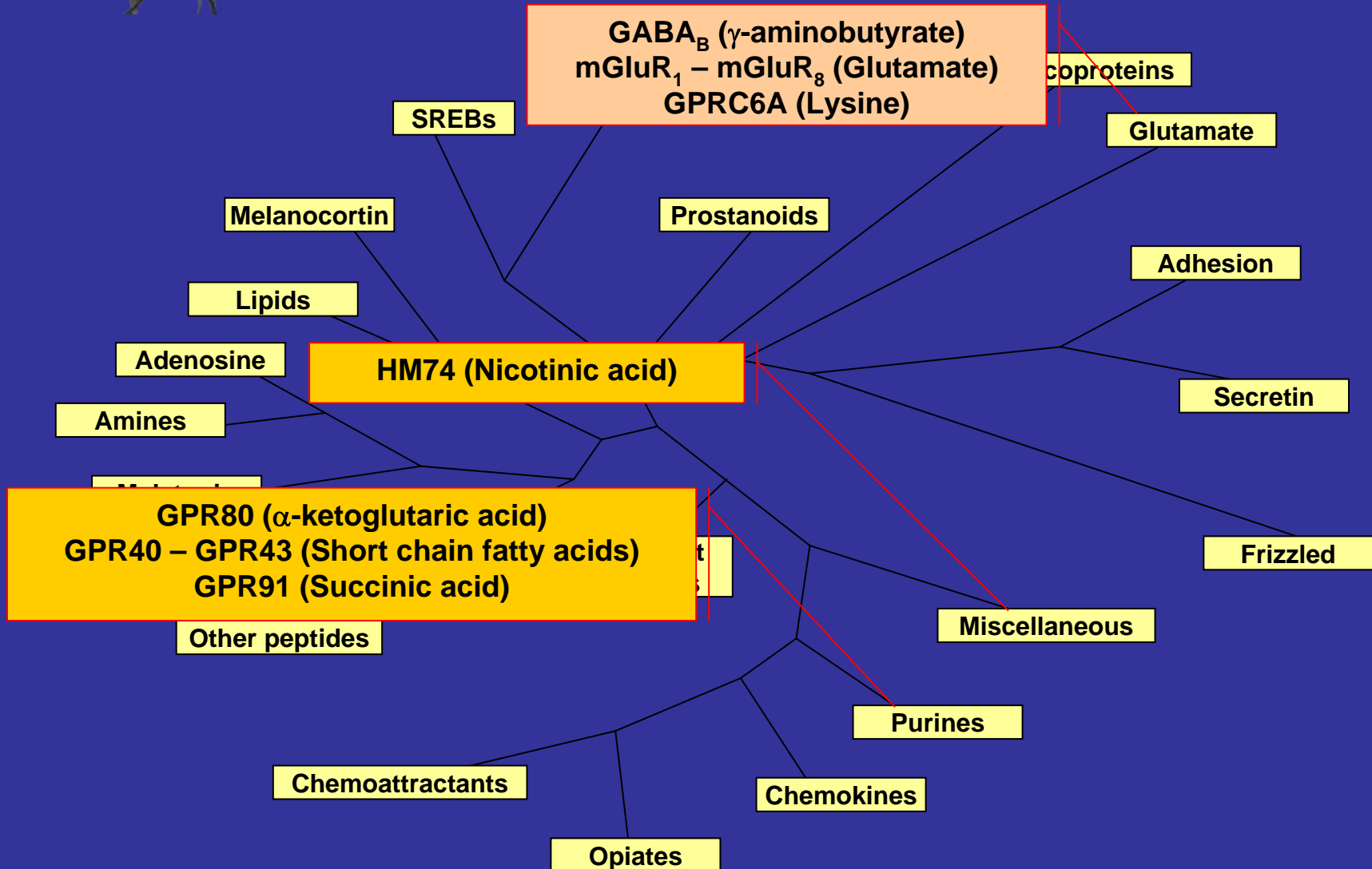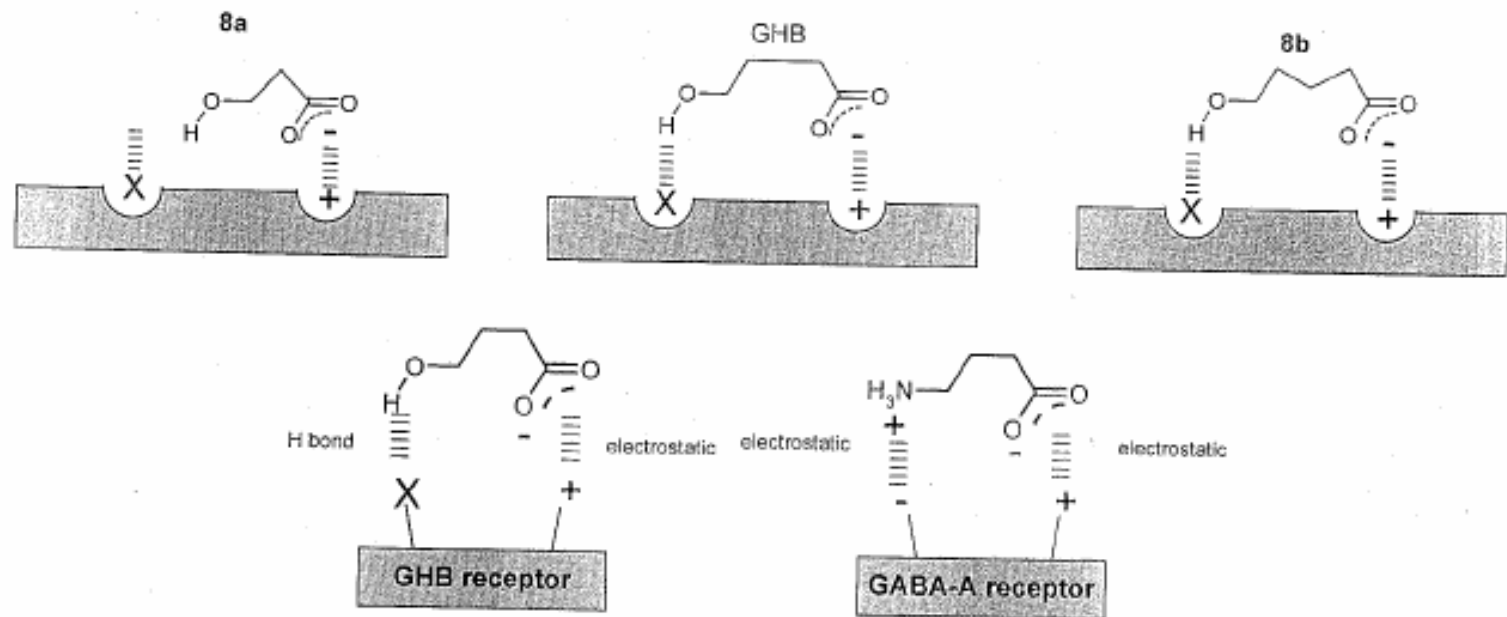
Arbre phylogénétique

Opsins

Glycoproteins

Glutamate

SREBs

Melanocortin

Acetylcholine Muscarine ($M_1 - M_5$)
Dopamine ($D_1 - D_2$)
Histamine ($H_1 - H_4$)
Noradrenaline ($\alpha_{1A} - \beta_1$)
Serotonine (5HT)
Trace amines ($TAR_1 - TAR_5$)

...sion

Lipids

Adenosine

...ecretin

Amines

Melatonin

Vasopeptides

Brain-gut peptides

Frizzled

Other peptides

Miscellaneous

Purines

Chemoattractants

Chemokines

Opiates

Récepteurs de Ligands type RFamide

Opsins

Glycoproteins

SREBs

Glutamate

Melanocortin

Prostanoids

$NPFF_1 - NPFF_2$
$NPY_1 - NPY_5$
GPR10
GPR54
GPR103

Lipids

Adenosine

Amines

Melatonin

Frizzled

Vasopeptides

Brain-gut peptides

Other peptides

Miscellaneous

Purines

Chemoattractants

Chemokines

Opiates

Récepteurs de Ligands non peptidiques type Acide Carboxylique

GABA$_B$ (γ-aminobutyrate)
mGluR$_1$ – mGluR$_8$ (Glutamate)
GPRC6A (Lysine)

SREBs

coproteins

Glutamate

Melanocortin

Prostanoids

Adhesion

Lipids

HM74 (Nicotinic acid)

Adenosine

Secretin

Amines

GPR80 (α-ketoglutaric acid)
GPR40 – GPR43 (Short chain fatty acids)
GPR91 (Succinic acid)

Frizzled

Other peptides

Miscellaneous

Purines

Chemoattractants

Chemokines

Opiates

Schematic representations of ligand - receptor interactions for both GHB and GABA.

Fig. 4.17. Binding of acetylcholine to the nicotinic receptor: hydrophobic groups bind two methyl groups, glutamate forms an ionic bond with the ammonium ion, and histidine holds the ester carbonyl through hydrogen bonding. The acetyl methyl will bind preferentially to the muscarinic receptor.
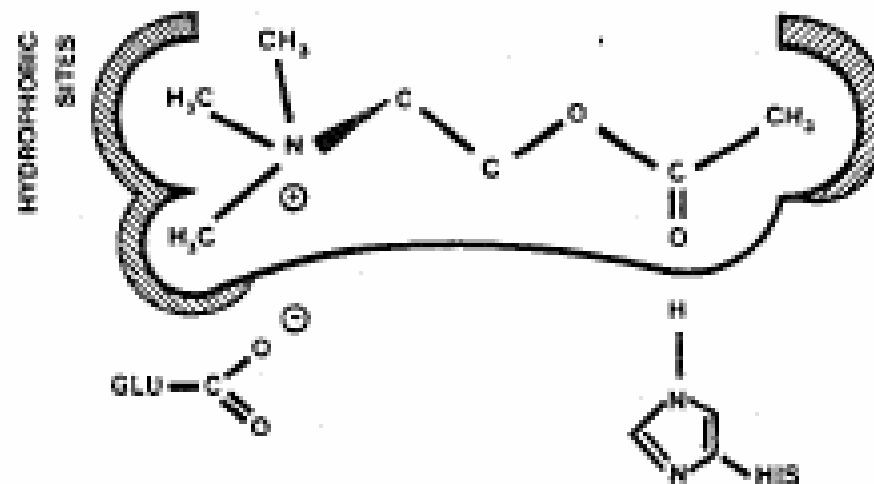
Role a specific Arg residue
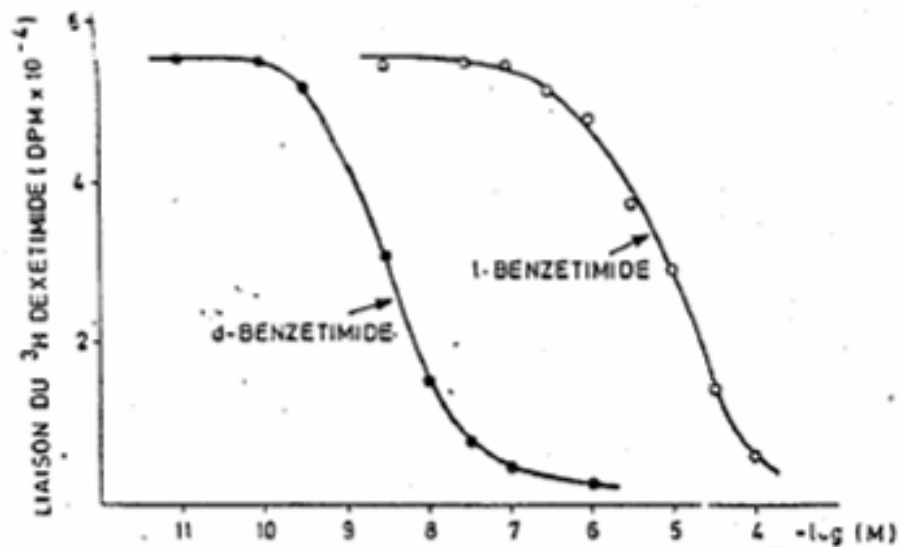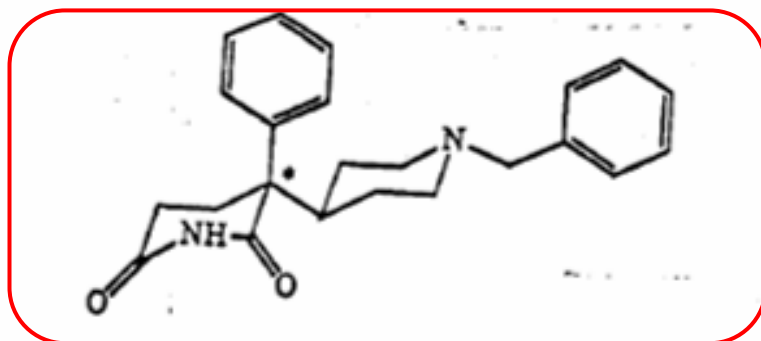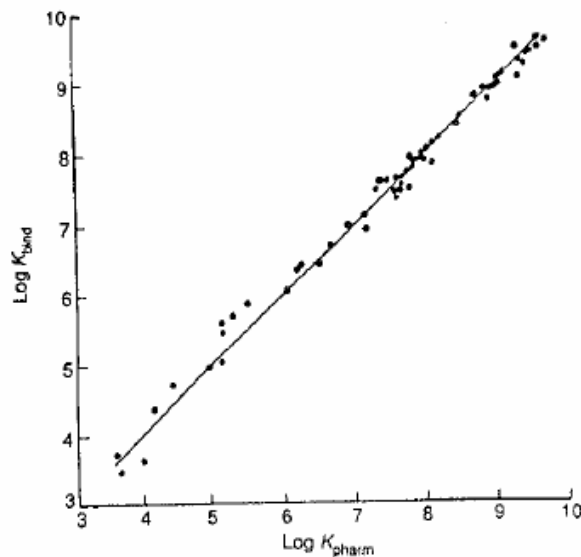in protein - ligand interactions
(example of HLA-B27 receptor)
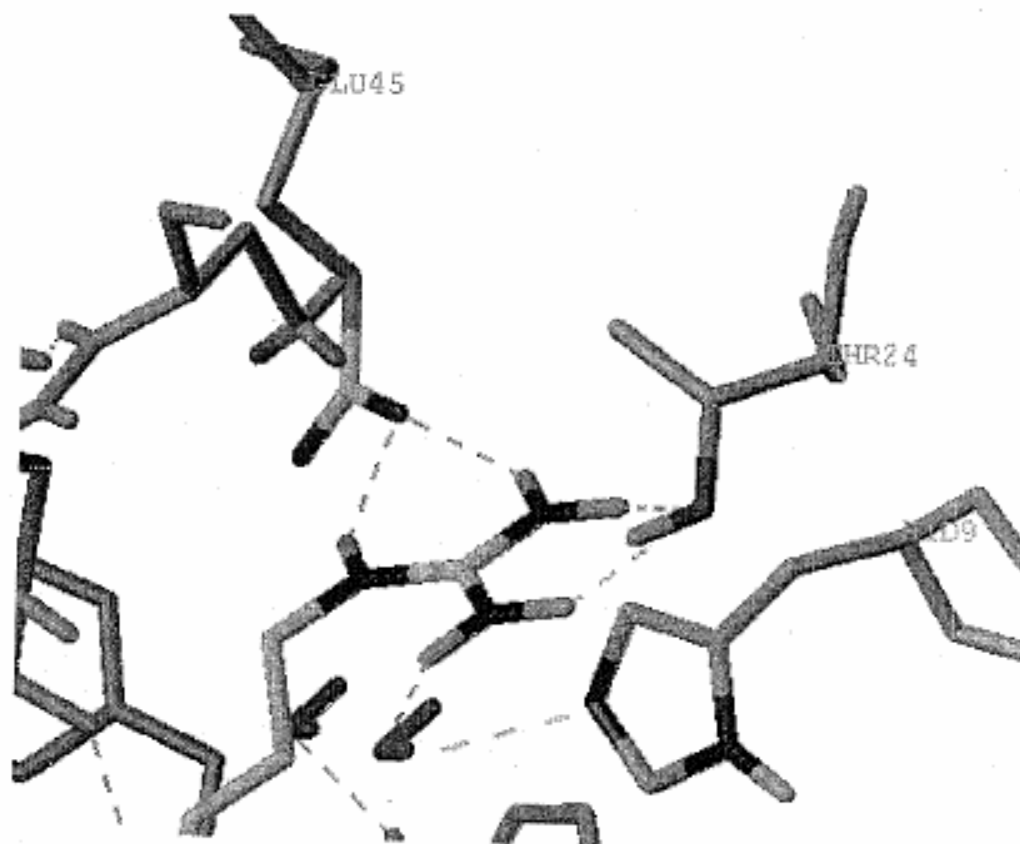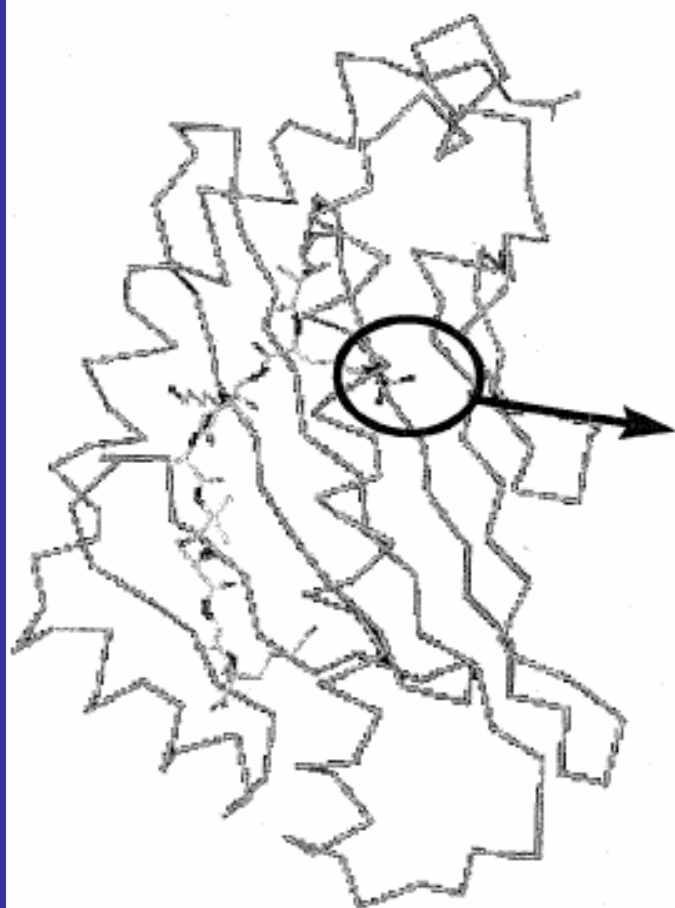
Figure 2 : Courbe d'inhibition du binding au dexétimide-H³ par les 2 isomères du benzétimide (dexétimide et lévitimide) en utilisant un homogénat total de noyau caudé de rat.

Figure 8. Correlation between the binding affinities of 60 muscarinic antagonists for rat cerebral cortical muscarinic receptors and their pharmacological potencies in antagonizing muscarinic contraction of the longitudinal muscle of the guinea-pig ileum. Subtype-selective antagonists deviate significantly from this correlation.
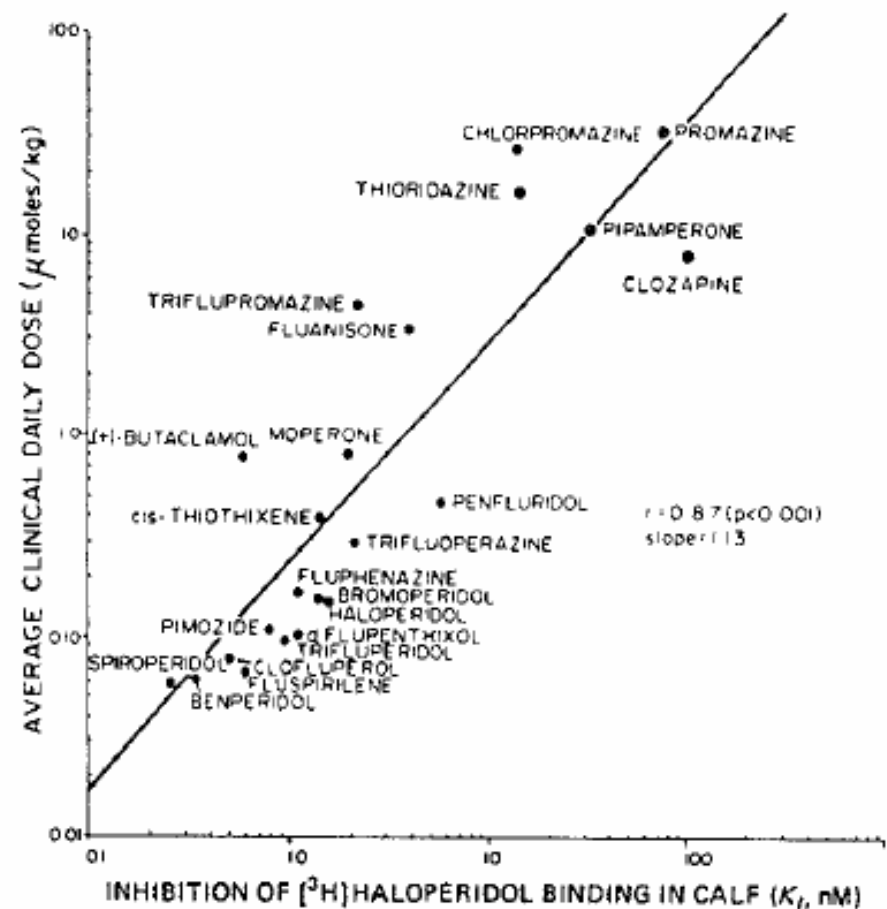
Fig. 4.31. Correlation of neuroleptic drug binding with average clinical dose. (Reproduced by permission from Creese et al. (1976), *Science 194*: 546)

Trends in Pharmacological Sciences

Rat

Can animal models really predict anxiety?

HOW PREDICTIVE ARE animal models of anxiety? This question, posed by Dr Colin Gardner at a recent meeting

Table I. Drugs reported to enhance sexual function

Drug	Libido	Male performance Erectile capacity	Male performance Ejaculatory function	Female performance (subjective self-assessment)
L-DOPA	+	O	O	O
Bromocriptine	+	O	O	O
p-Chlorophenylalanine (PCPA)	+	O	O	O
Yohimbine	O	O	O	O
L-Tryptophan	O	O	O	O
Luteinizing hormone	+	O	O	O
Ethanol	+	↓	↓	+/−
Amphetamine	+	−	+/−	+/−
Amyl nitrite	+	↑ ↓	O	O
Cannabis	+/−	↑ ↓	O	+/−
Cocaine	+	↓	↓	+/−
Methaqualone	+	↓	O	+
Placebos	+	↑	↑	+

Key: O: no change; + or ↑ : increase; − or ↓ : decrease. Data from Refs 1 and 3.

STRATEGIES GUIDING MEDICINAL CHEMISTRY

- Efficient criteria for the choice of reference compound(s)
(*endogenous or synthetic substances, agonists or antagonists, profiling*)

- A clear and efficient strategy
(*fast chemical pathways, go/no go decisions*)

- Rapid access to a first set of derivatives

↓

First SAR analysis

new set of derivatives   structural analogues
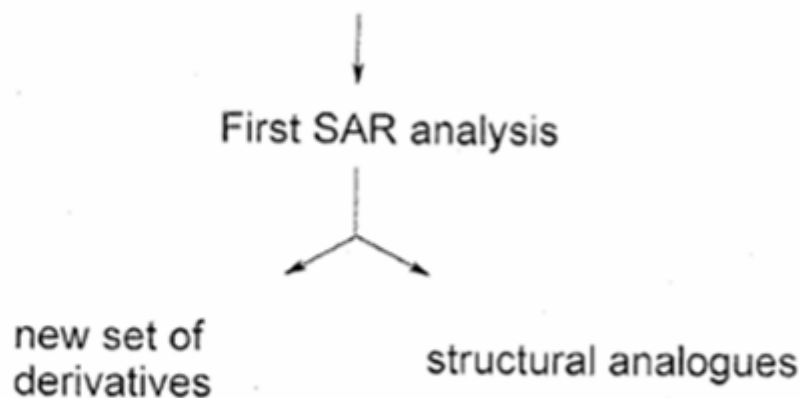
Pharmacological tools

. Selective agonists or antagonists acting at new receptors

- G.Protein coupled receptors (GPCR)
- ion channels
- enzyme coupled receptors

.Competitive or non competitive enzyme inhibitors.

.Radio-ligands (^{3}H, ^{125}I, ^{14}C,)

. Fluorescent probes

HOW TO PROCEED

1. Which disease (CNS, cardio-vascular, pulmonary, obesity, cancer, asthma, etc …).

2. Localization (tissue, cell type), identification, and characterisation of a target (or a cascade of events) involved in a physiological process in the relation with the disease).

3. Hypotheses (molecular + physiological).

4. In vitro and in vivo models.

START FROM SCRATCH
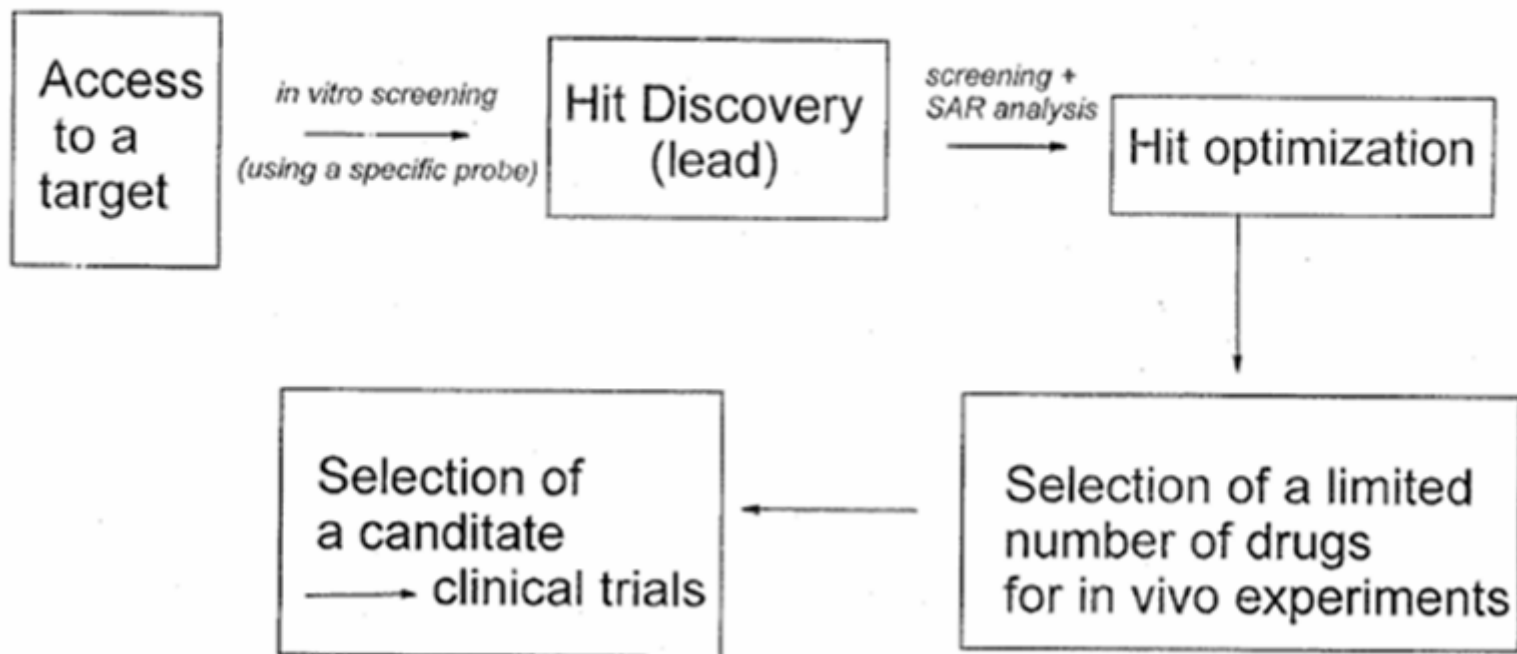
The medicinal chemistry challenge

First step: In vitro **screening for drug optimization**

I Start from a hit (lead) compound (rational approach)

- a endogenous ligand
- an already known pharmacological agent ("me too ")

II Random screening (serendipity)

(High throughput screening techniques)

- HOMOLOGY
- ISOSTERY
- SUBSTITUTION
- ADDITIONAL INTERACTIONS
- SIMPLIFY A COMPOUND
- RIGIDIFY A COMPOUND
- ARIENS THEORY (how to make an antagonist)

Activity

Chain length
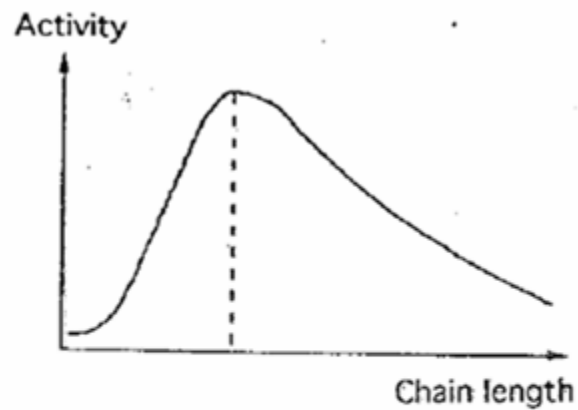
Nonsymmetrical curve with a maximum activity peak.
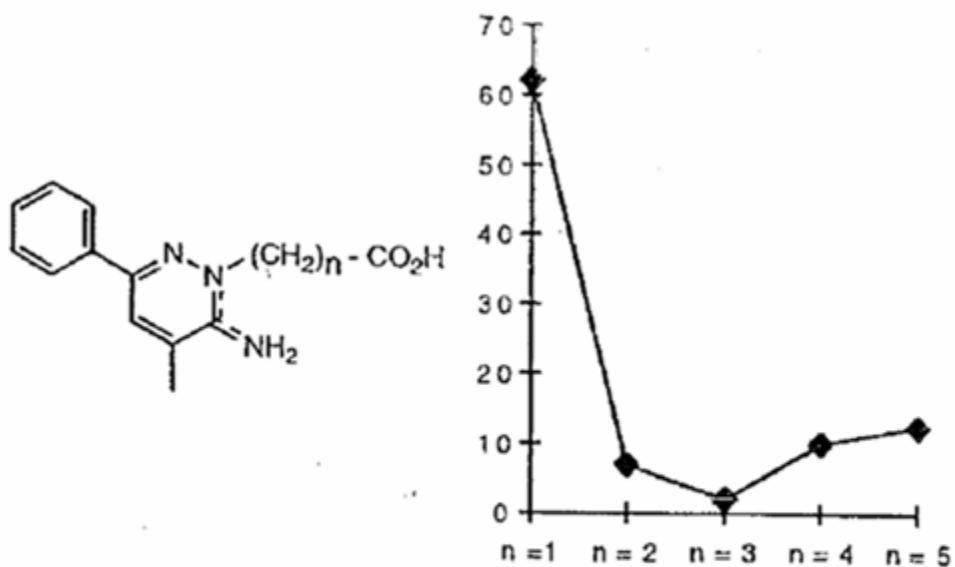
Affinity of GABA$_A$ antagonists for the GABA$_A$ receptor site.[17]

ISOSTERY

$$- O \cdots\cdots - NH - \cdots\cdots - CH_2-$$
$$-S-$$

$$Cl \cong CN \cong SCN \quad etc$$
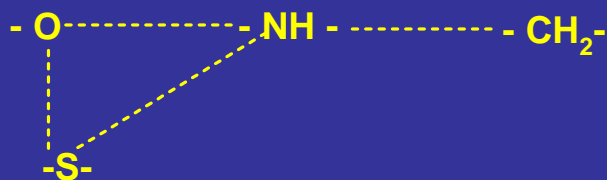
Ring equivalents

Electronic parameters govern the nature and the quality of ligand–receptor or ligand–enzyme interactions. The relevant parameters will be inductive or mesomeric effects, polarizability, pK_a, capacity to form hydrogen bonds, etc. Despite their very different substituents in the *meta* position, the two epinephrine analogues (Fig. 13.22) exert comparable biological effects: they are both β-adrenergic agonists. In fact the key parameter resides in the very close pK_a values.[87]

$pK_a = 9.6$ $pK_a = 9.1$
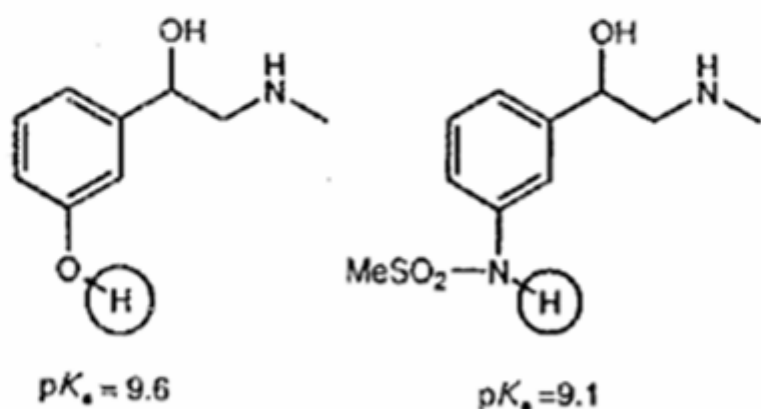
Fig. 13.22 An example of bioisosterism, or nonclassical isosterism; the methylsulphonamide substituent has comparable acidity to the phenolic hydroxyl group.[76]
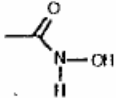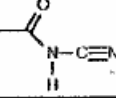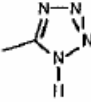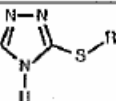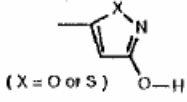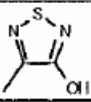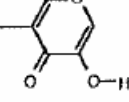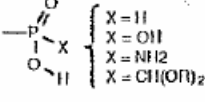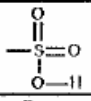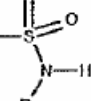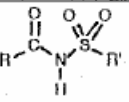
BIOISOSTERIC COMPOUNDS
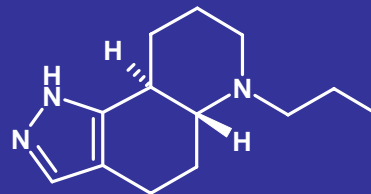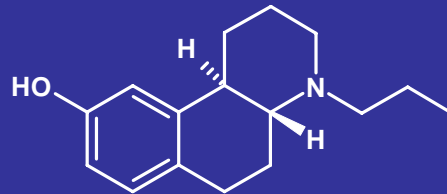
**Selective D2 agonists**

ISOSTERY: Carboxylic acid isosteres

Structure	Name	Description	Reference
	HYDROXAMIC ACIDS	High chelating power	Almquist et al.[27]
	ACYL-CYANAMIDES	Mainly academic interest	von Kohler et al.[28] Shirota et al.,
	TETRAZOLES	Very popular Great number of publications. Recent in use. pKa = 6,6 to 7,2	Bovy et al.[29] Marshall et al.[30]
	MERCAPTOAZOLES + Sulfinylazoles + Sulfonylazoles	Phosphonate isosteres pKa mercapto: 8,2-11,5 pKa sulfinyl: 5,2-9,8 pKa sulfonyl: 4,8-8,7	Chen et al. [12]
(X = O or S)	ISOXAZOLES ISOTHIAZOLES	GABA and glutamic acid analogues	Krogsgaard-Larsen et al. [31] Krogsgaard-Larsen[32]
	HYDROXY-THIADIAZOLE	Isoxazole isostere pKa # 5	Lunn et al.[33]
	HYDROXY-CHROMONES	Kojic acid derivatives : As GABA agonists	Atkinson et al.[34]
X = H X = OH X = NH2 X = CH(OH)₂	PHOSPHINATES PHOSPHONATES PHOSPHONAMIDES	Many examples in the glutamate antagonist series and in the GABA$_B$ antagonists	Froestl et al[35]
	SULPHONATES	Sulphonic analogues of GABA and glutamic acid	Rosowsky et al., 1984
	SULPHONAMIDES	Weak acids, used rather as equivalents of phenolic hydroxyls: catecholamine analogues	von Kohler et al.[28]
	ACYL-SULPHONAMIDES	Glycine GABA β-alanine antiatherosclerotics pKa # 4,5	Drummond & Johnson[36] Albright et al.[37]

3. A NEW CLASS OF GABA_B RECEPTOR AGONISTS AND ANTAGONISTS

By replacing the carboxylic acid moiety of GABA with various phosphinic acid residues, a new series of potent GABA_B ligands was discovered *(Fig. 2)*.
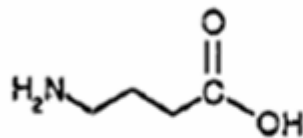
GABA
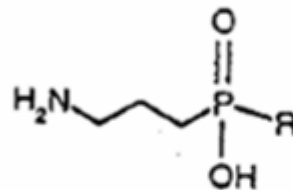
Fig. 2

R = H	CGP 27 492	agonist
R = Me	CGP 35 024	agonist
R = Et	CGP 36 216	antagonist
R = CH(OEt)$_2$	CGP 35 348	antagonist
R = n-Bu	CGP 36 742	antagonist
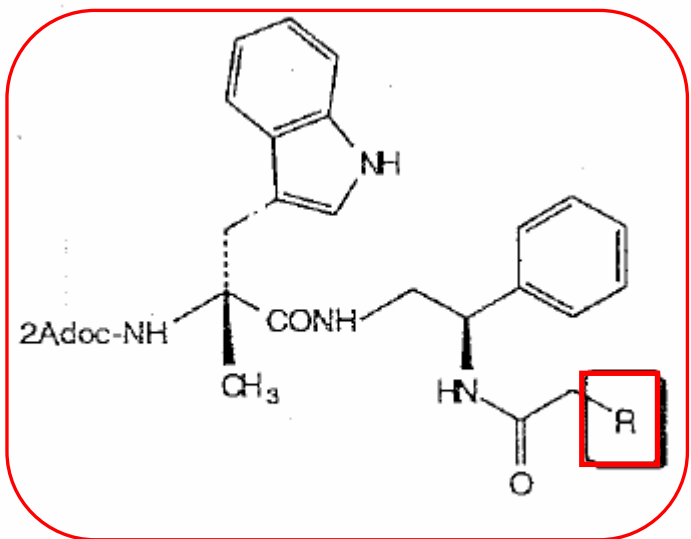
R	IC$_{50}$ (nM) CCK-B	IC$_{50}$ (nM) CCK-A	A/B ratio	pK_a
$-CH_2-COOH$	1.7	4500	2500	5.6
Charge-distributed monoanionic acid mimics				
	6.0	970	160	5.4
	2.6	1700	650	6.5
	2.4	620	260	4.3
	2.5	680	270	>9.5
	16	850	53	>9.5
	4.3	660	150	7.7
	1.7	940	550	7.0

OPTIMISATION STRUCTURALE PAR EFFET DE SUBSTITUTION
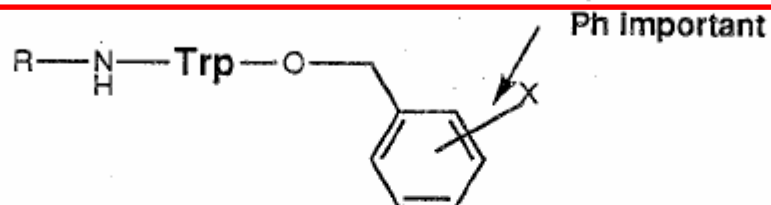
R	X	Déplacement de ^{125}I - SP CI_{50} (nM)
Et	H	3 800
H	H	> 10 000
Boc	H	413
Boc	3,5 $(Me)_2$	133
Ac	3,5 $(Me)_2$	67
Ac	3,5 $(CF_3)_2$	1,6
(morpholine)$N-(CH_2)_3$	3,5 $(CF_3)_2$	0,17 soluble/H_2O
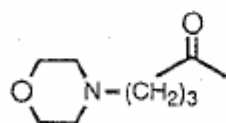
METHYL EFFECTS
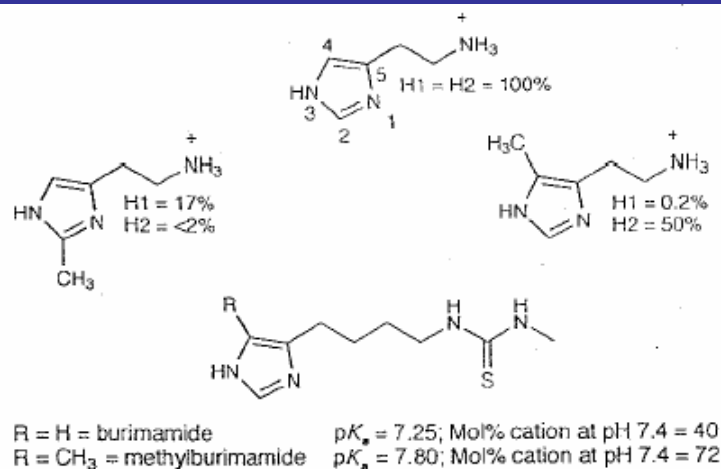
Methyl effects in histamine derivatives
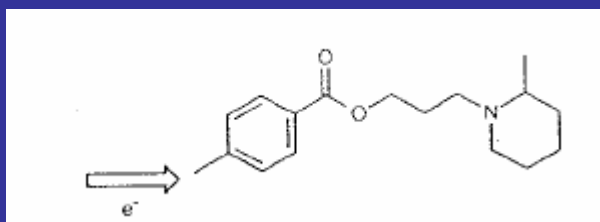
Stabilization of the ester function thanks to an inductive effect of the p-methyl group.

1. Oxidation of the methyl group

The oxidation of the methyl group usually continues up to the carboxyl stage (Fig. 17.10). This is observed for camphor, for 2-methylpyridine and for the drugs tolbutamide and alpidem, explaining the relatively short half-lives of these latter compounds.
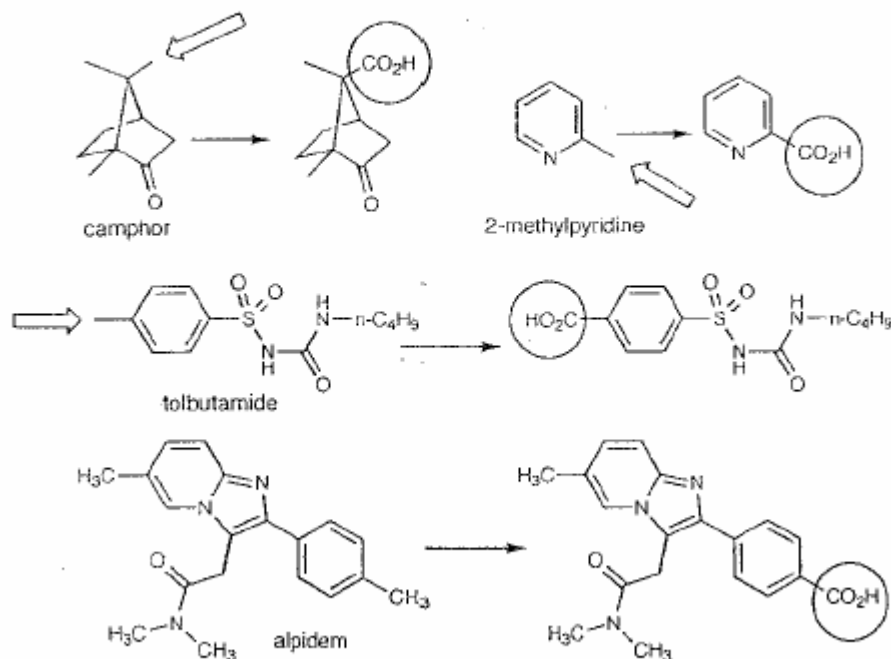
Examples of oxydation of methyl to carbonyl groups

Antagonists of benzodiazepines
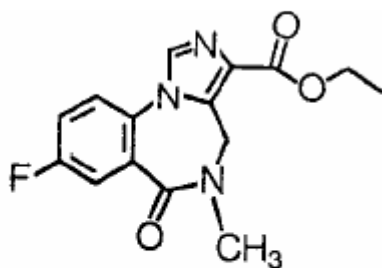
Ro 15–1788
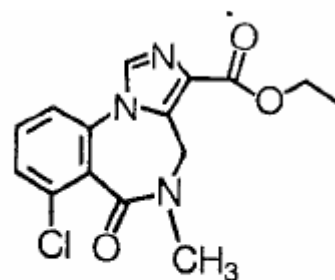
Ro 15–3505

Benzodiazepine agonist
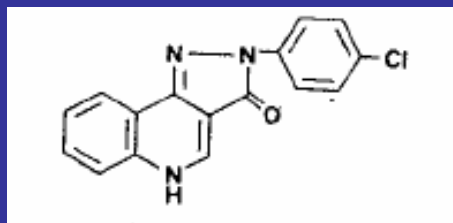
Benzodiazepine antagonist

CGS 8216
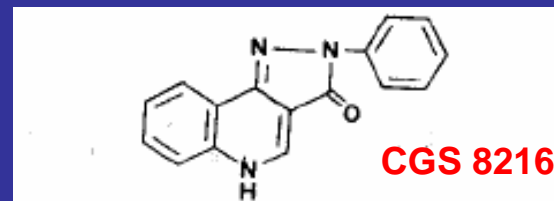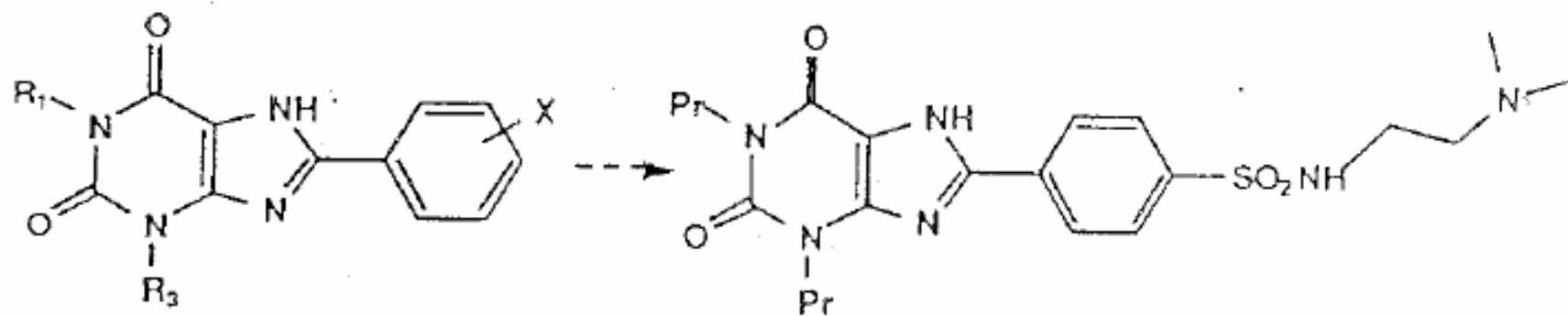
Design of water-soluble xanthine antagonists
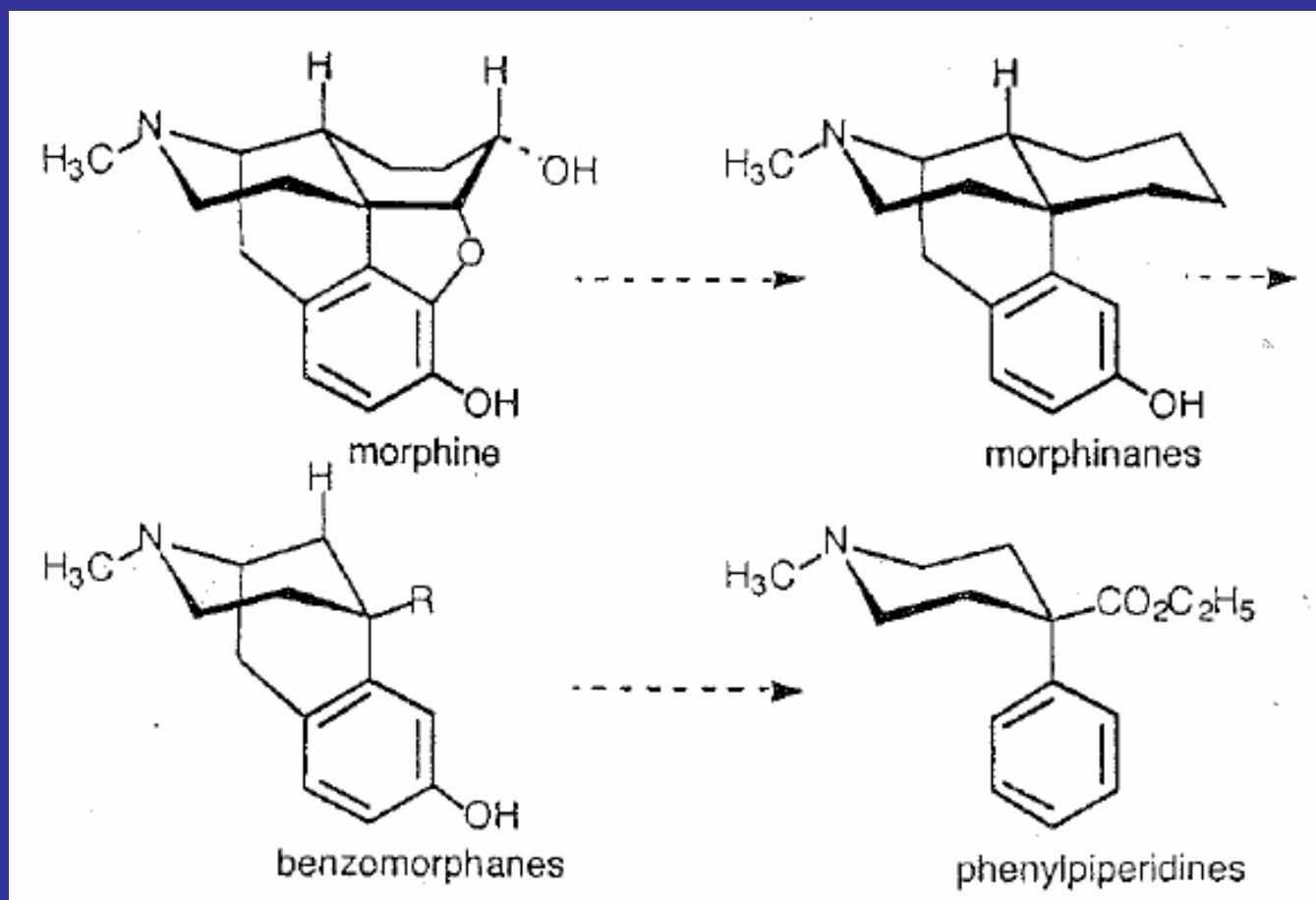
Progressive simplification of the morphine molecule
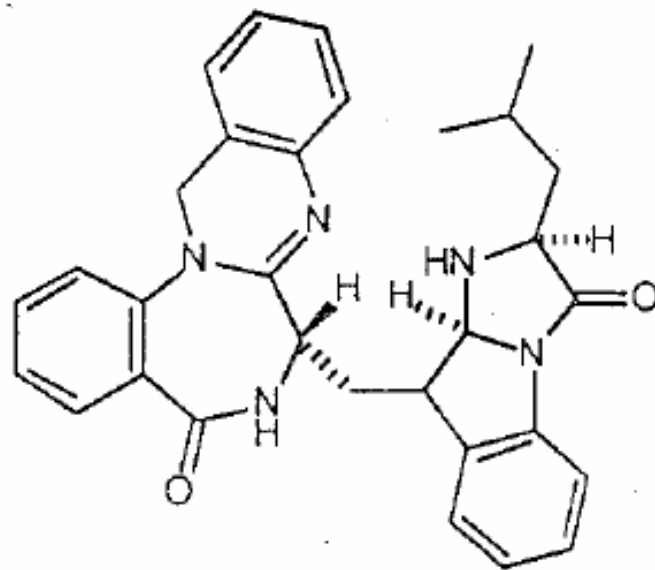
asperlicine
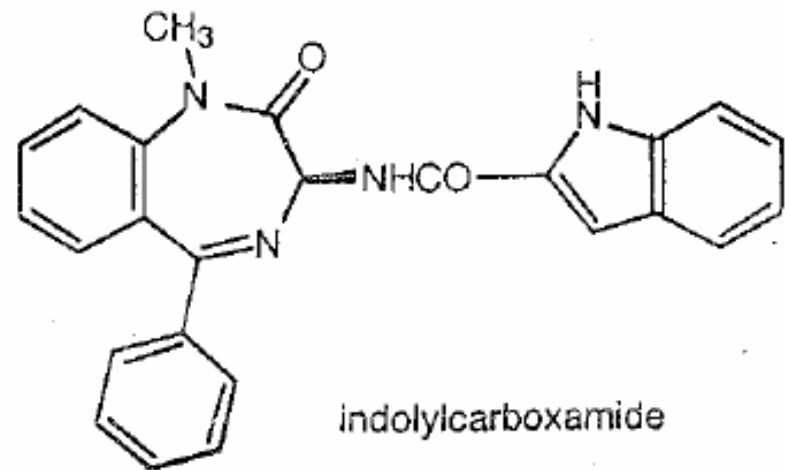
indolylcarboxamide

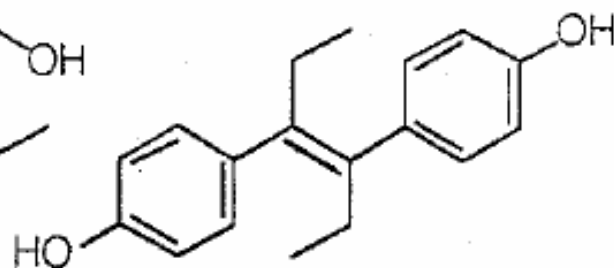Productive disjunction of the asperlicin molecule
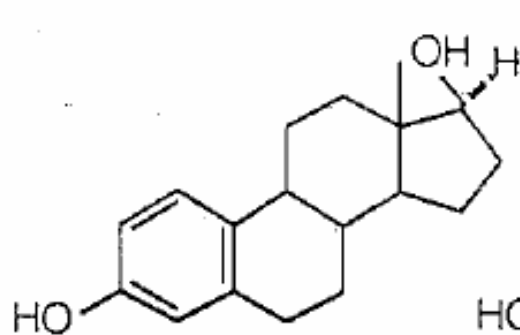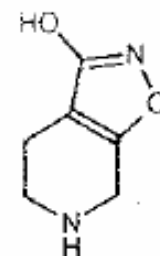
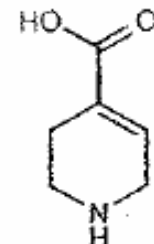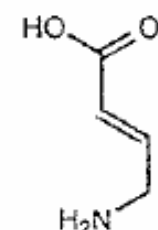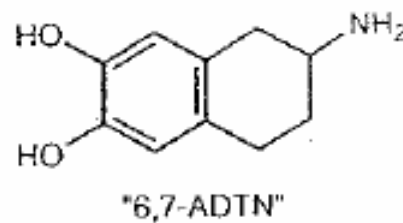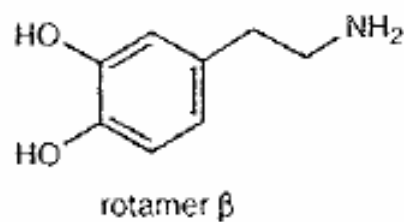estradiol
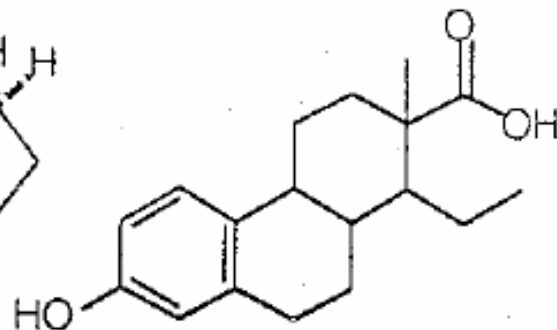
bisdehydro-doisynolic acid
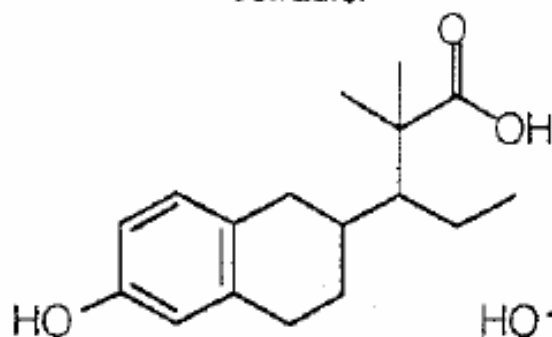
allenoestrol

diethylstilboestrol

Open analogues of estradiol

rotamer α

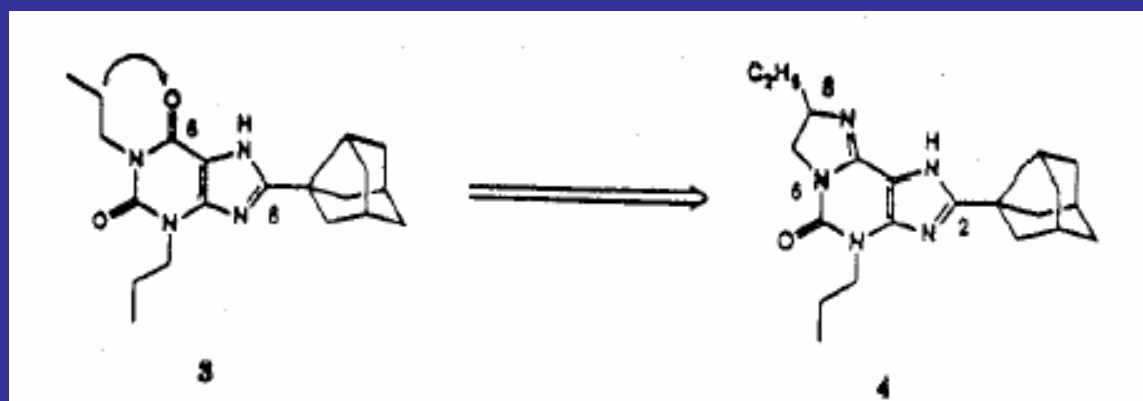"5,6-ADTN"

rotamer β

"6,7-ADTN"
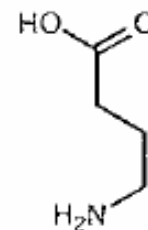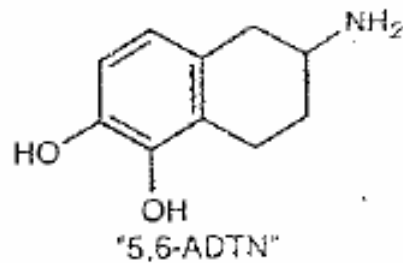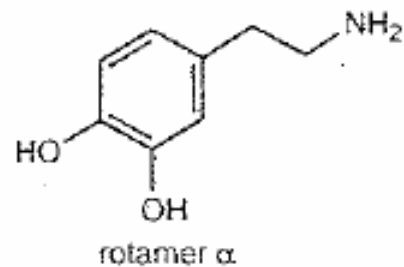
Ring-closed analogues of the two rotamers of dopamine.

GABA

trans - 4-amino-
crotonic acid

isoguvacine

THIP

GABAergic agonists.

Typical types of rigidification
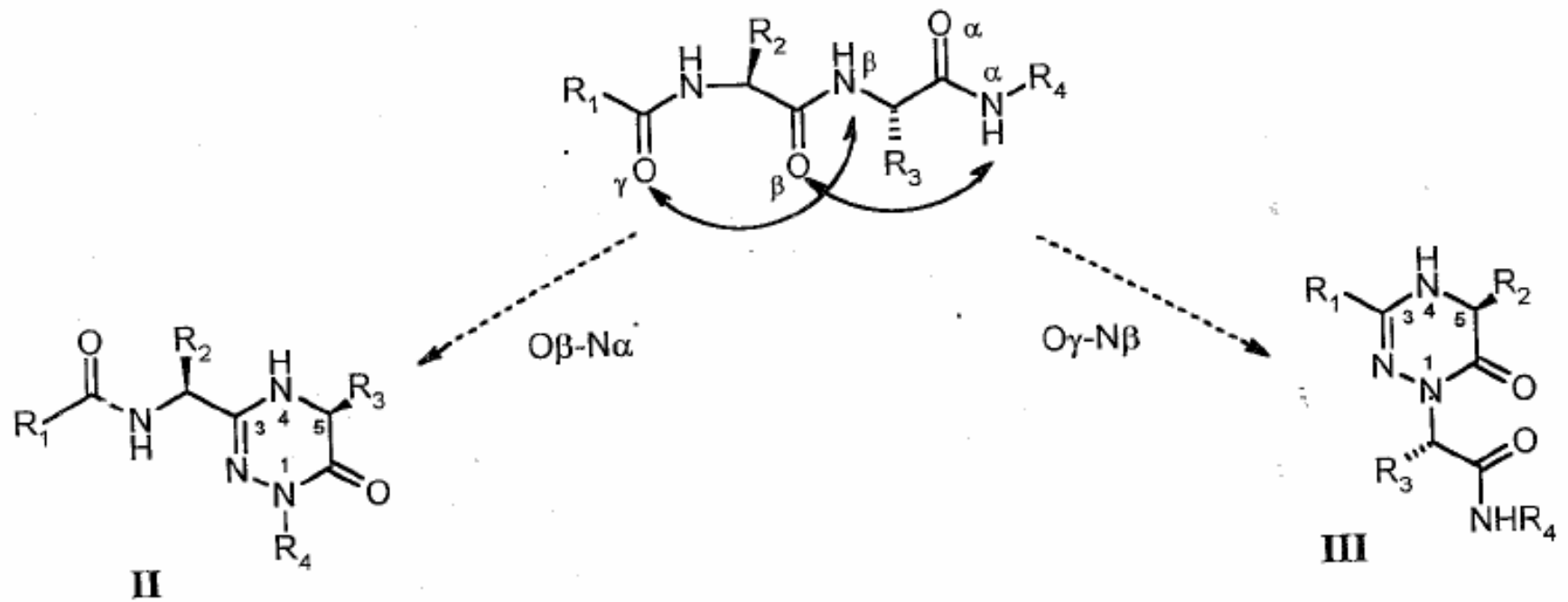
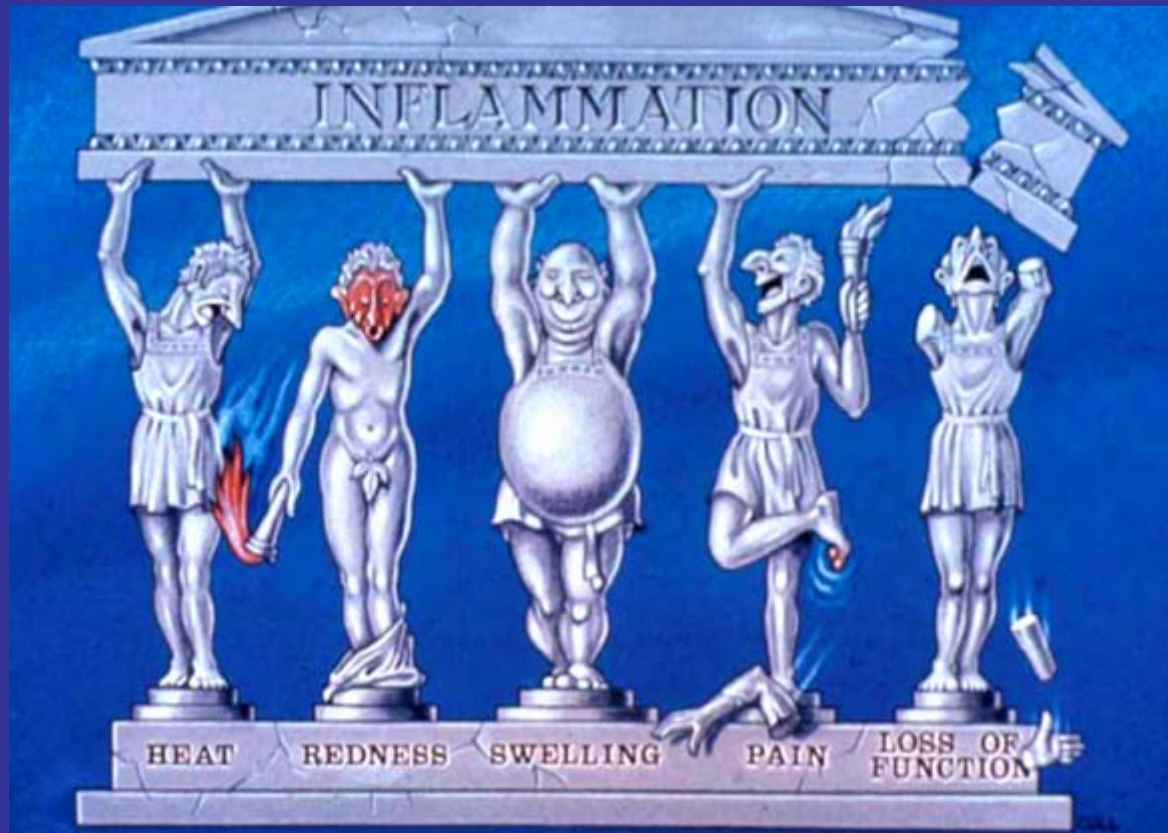R_1, R_4 = alkyl, aryl, function or α-aminoacid

Table I. A_1 and A_2 Receptor Binding and Water Solubility of Adenosine Antagonists

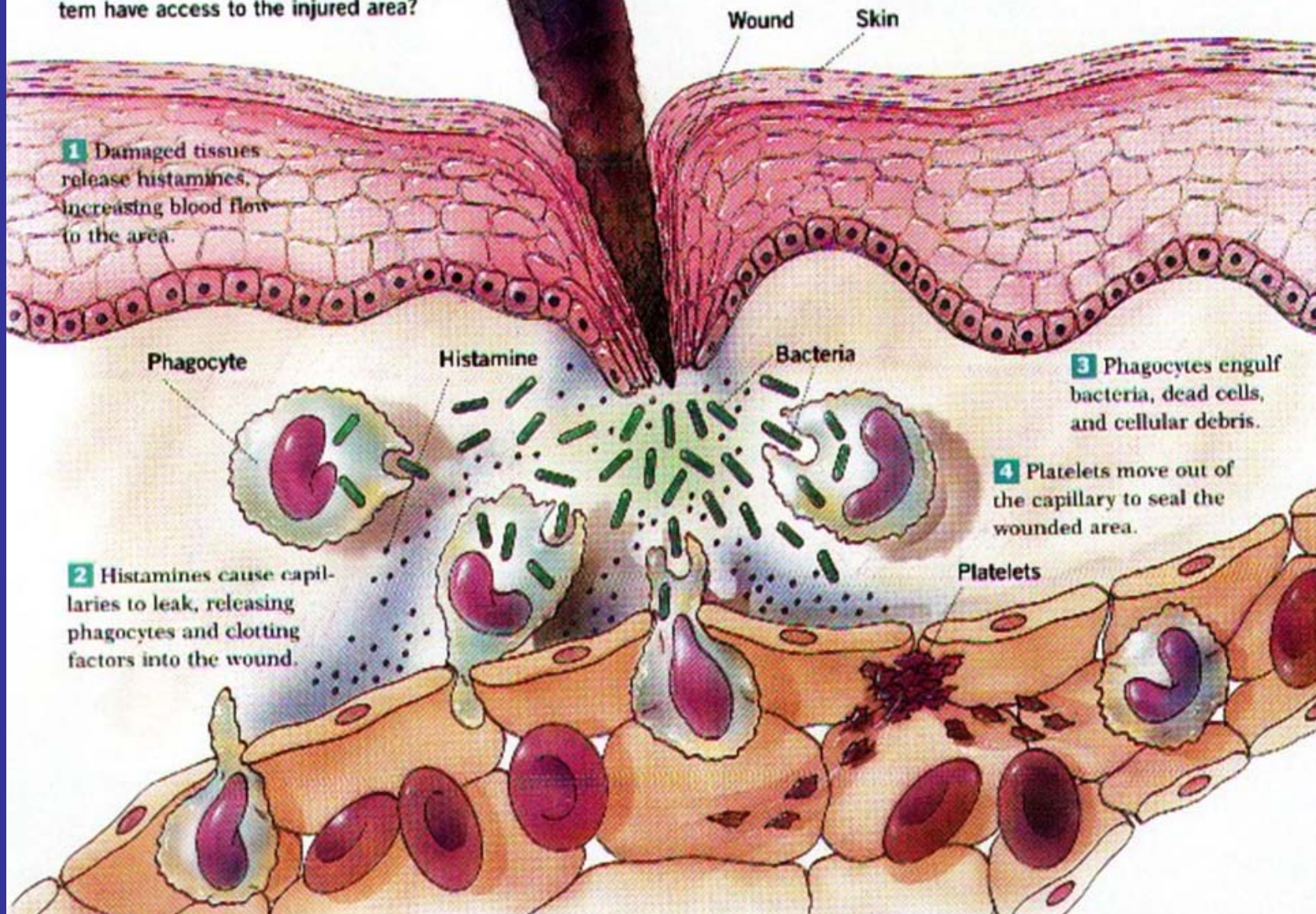| compd | K_i,[a] nM | | K_i ratio A_2/A_1 | solubility,[b] µg/mL | |
	A_1	A_2		water	saline
1	6.4 ± 0.35	590 ± 48	92	3.3	NT
3	1.3 ± 0.12	430 ± 30	290	0.5	0.6
4	5.7 ± 0.51	330	58	3200	990
8(R)	2.7 ± 0.09	290	107	3000	NT
9(S)	120	250	2.1	2900	NT

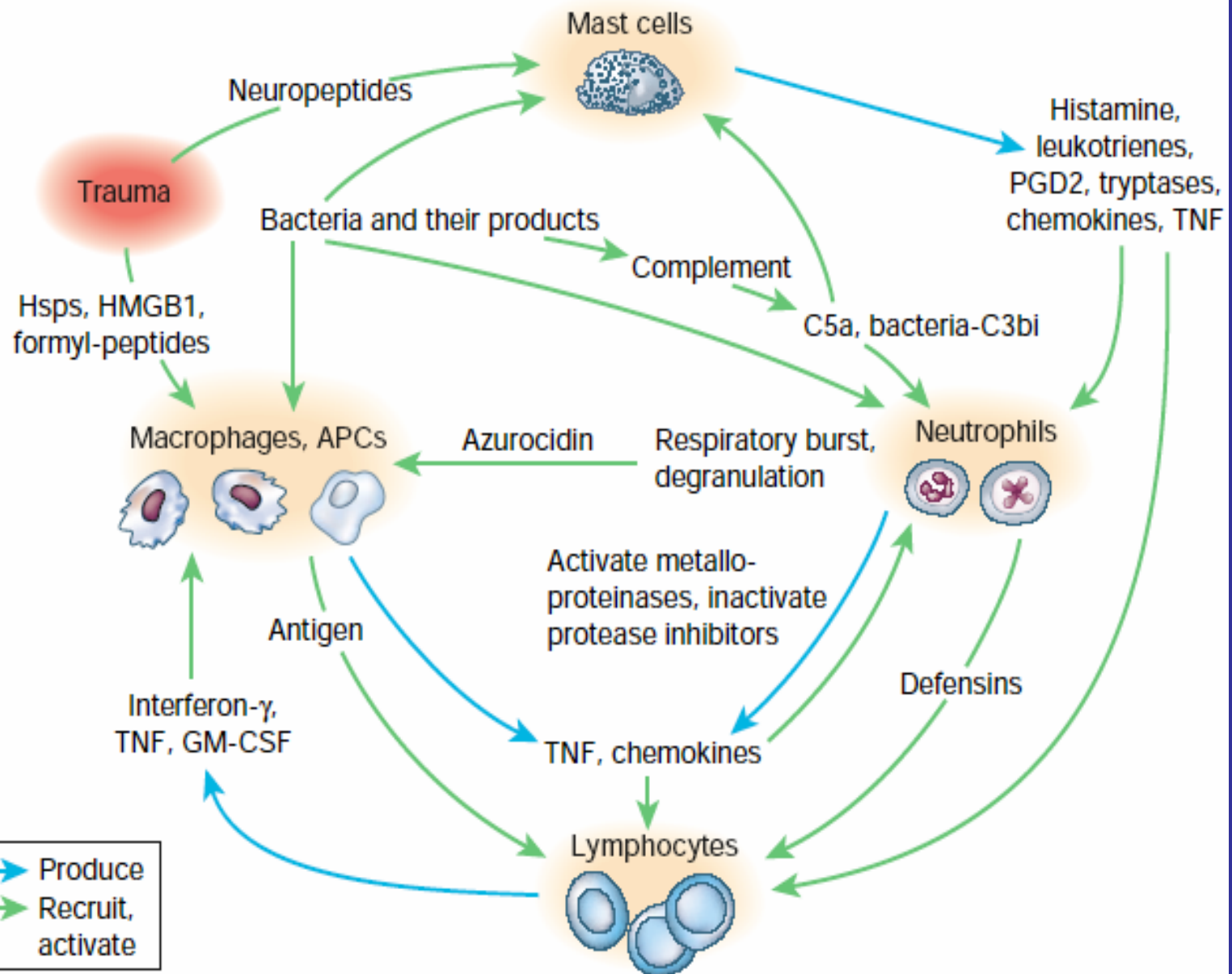# DEVELOPMENT OF NOVEL ANTI-INFLAMMATORY COMPOUNDS

The inflammatory response is a body's second line of defense against invasion by pathogens. Why is it important that clotting factors from the circulatory system have access to the injured area?
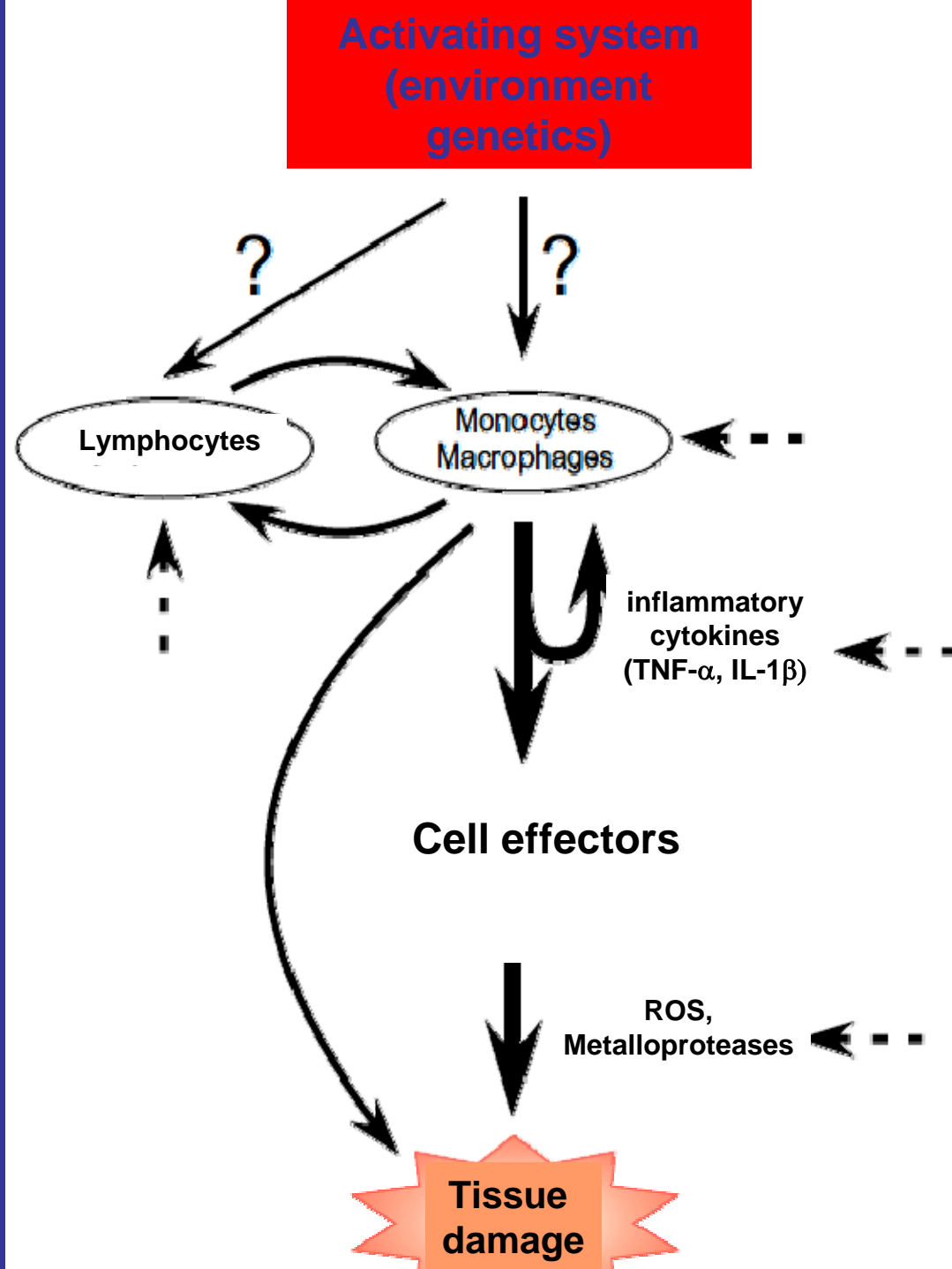
THE INFLAMMATORY RESPONSE

Wound

Skin

1 Damaged tissues release histamines, increasing blood flow to the area.

Phagocyte

Histamine

Bacteria

3 Phagocytes engulf bacteria, dead cells, and cellular debris.

4 Platelets move out of the capillary to seal the wounded area.

Platelets

2 Histamines cause capillaries to leak, releasing phagocytes and clotting factors into the wound.

COMMON MECHANISMS DEALING WITH CHRONIC INFLAMMATORY DISEASES

- asthma,
- rheumatoïd arthritis,
- multiple sclerosis,
- Alzheimer disease,
- Crohn's disease,
- atopic dermatitis,
- septic shock

Activating system (environment genetics)

Lymphocytes

Monocytes Macrophages

inflammatory cytokines (TNF-α, IL-1β)

Cell effectors

ROS, Metalloproteases

Tissue damage

Use of anti-tumour necrosis factor agents in inflammatory bowel disease

Inhibitors of *de novo* TNF-α synthesis

- α-Phthalimido-glutamide (thalidomide, Grünenthal/Cellgen): inhibits TNF production in vitro but is unlikely to inhibit TNF production in vivo. In diseases other than Crohn's disease thalidomide is not a potent TNF inhibitor in vivo.

- ISIS 104838 (OraSense, ISIS/Elan Pharmaceuticals): orally antisense-oligonucleotide directed against mRNA for human TNF -α.

- Oxpentifylline (Hoechst): PDE4 inhibitor, which has shown anti-TNF effects in vitro as well as in post-transplantation trials.

IN VITRO ACTION OF PDE 4 INHIBITORS

Bronchial smooth muscle	**Relaxation**
Mast cells	**Inhibition of antigen-induced mediator (histamine, LTC4) release**
Basophils	**Inhibition of antigen-induced mediator (histamine, LTC4) release**
Neutrophils	**inhibition of : respiratory burst, degranulation, PAF, LTB4 release**
Eosinophils	**inhibition of : respiratory burst, degranulation**
Endothelial cells	**Reduction of permeability**
Macrophages	**Inhibition of AA breakdown**
	Inhibition of cytokine (TNF-α) release
Monocytes	**Inhibition of cytokine (TNF-α) release**
Lymphocytes	**Inhibition of human cytotoxic T-lymphocyte activity**
	Inhibition of IL-2 release from T-lymphocytes
	Inhibition of T-cell blastogenesis
	Inhibition of IgE production from lymphocytes of atopic subjects

CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE1 TO PDE5) ISOFORMS

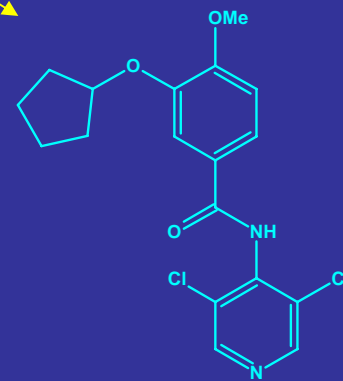| Isoforms	PDE1	PDE2	PDE3	PDE4	PDE5
Tissue species	Vascular smooth muscle	platelets	heart	vascular smooth muscle, brain, lung	platelets
Substrate	cGMP cAMP	cGMP cAMP	cAMP	cAMP	cGMP

DESIGN OF PDE4 INHIBITORS
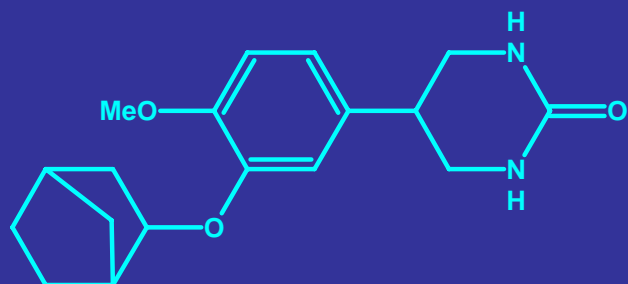
- **Starting from Rolipram**
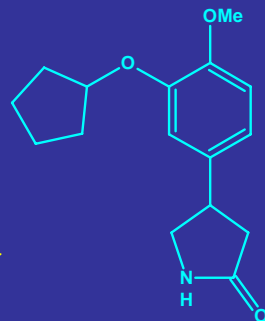
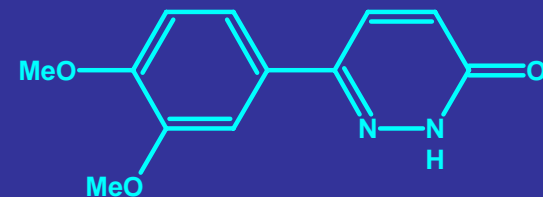Rolipram
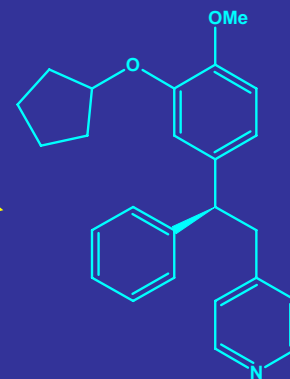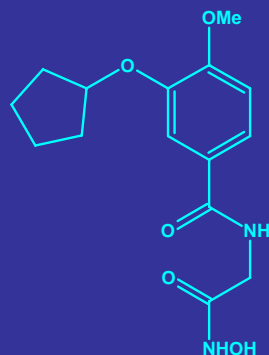
CDP840
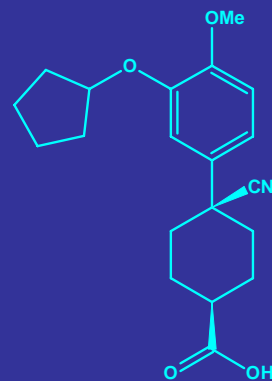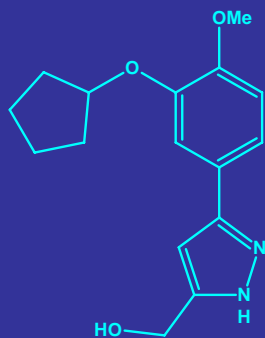
Ariflo

RP73401

DESIGN OF PDE4 INHIBITORS

• from pyrrolidones (Rolipram) to pyridazinones

Atizoram
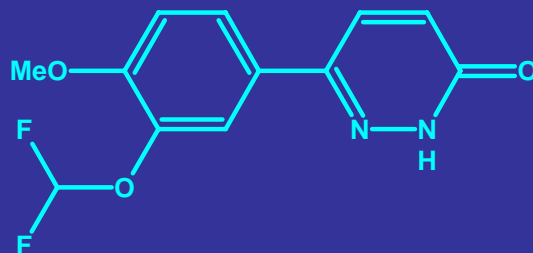
Zardaverine

Patent

Substituted 6-aryl pyridazones as PDE4 inhibitors

$R, R_1 = (CH_2)_n$ - Functional Group (FG)

Spacer

short (n=0-1)
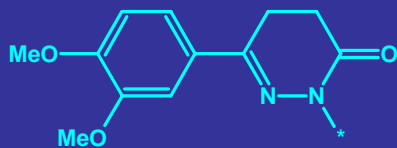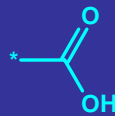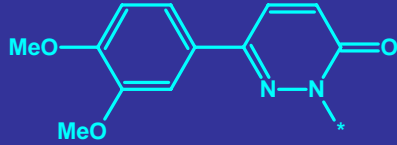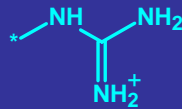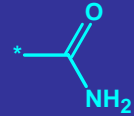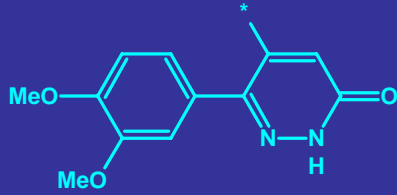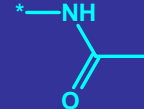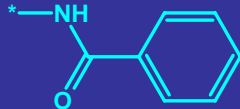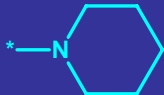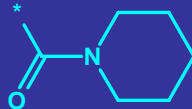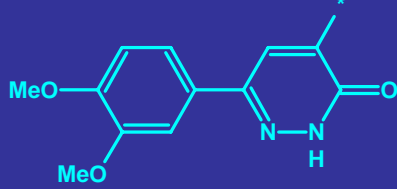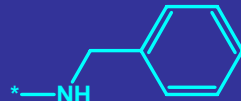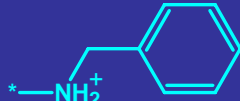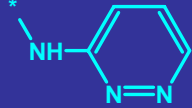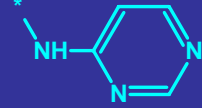
intermediate (n=2-4)

long (n=8-12)

additionnal

interaction
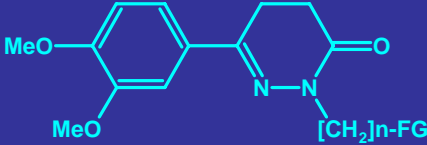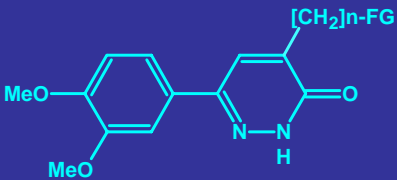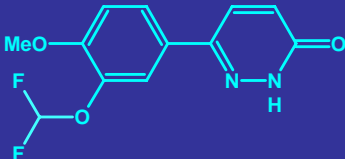
Molecular Fragments Used

Design of small-sized libraries by combinatorial assembly of linkers and functional groups to a given scaffold :Application to the structure-based optimization of a phosphodiesterase 4 inhibitor. Krier et al. *J.Med.Chem.,* submitted.

Zardaverine- Two further binding modes

PDB Code : 1XOR
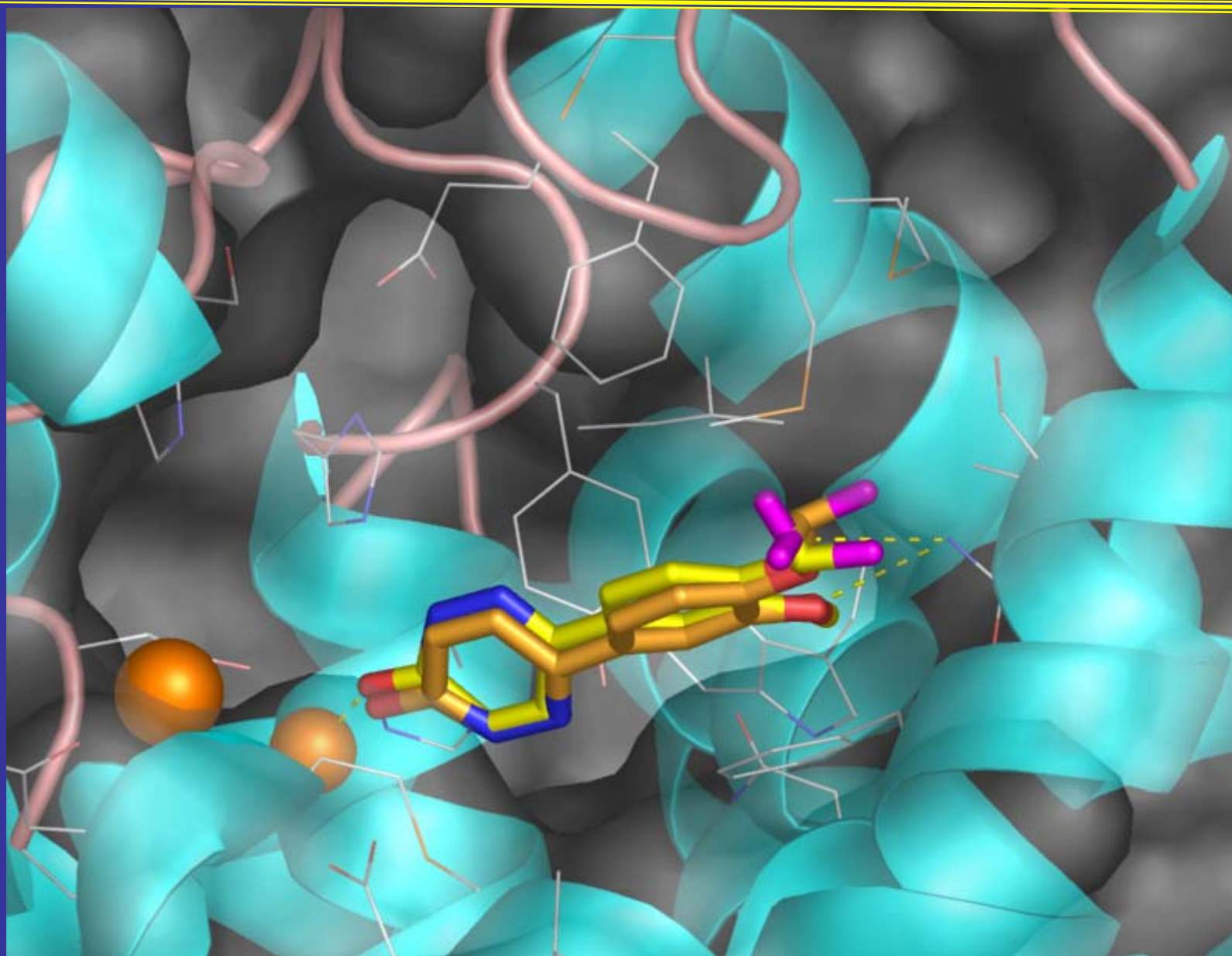
	FG	n	IC_{50}, nM
	H	0	2,000
	Ph	1	60
	Ph	3	20
	Ph	5	0.9
	Ph	6	80
	NH_2	6	20
	$NHC(NH)NH_2$	6	60,000
	Ph	1	8,000
	Ph	3	10,000
	Zardaverine		800

DESIGN OF PDE4 INHIBITORS

- **Rational approach**

cAMP

substrate

PDE4

5'-AMP
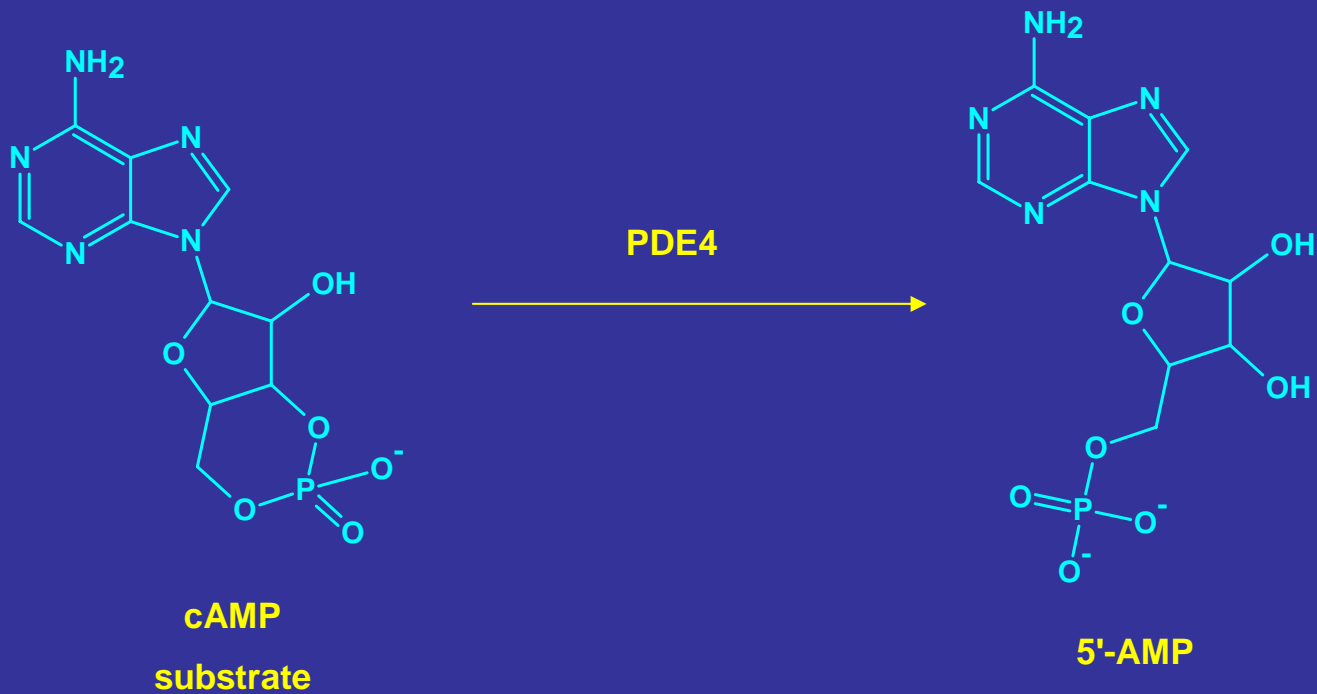
competitive inhibitor inactive
IC50 > 100 μM

BWA78U, a pharmacologically active adenine derivative
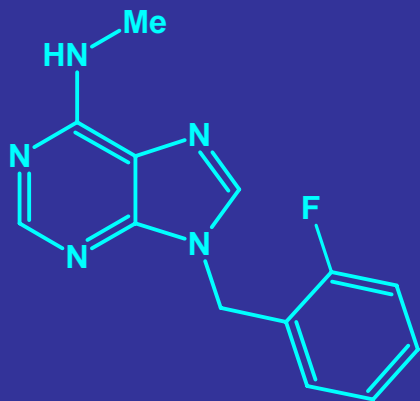
BWA78U

- **Anticonvulsant at 1-3 mg/Kg p.o. (electroshock, PTZ)**

- **Anxiolytic (non antagonized by flumazenil)**

- **Affinity for BZD receptors : IC_{50} = 13 μM**

- **Non active on adenosine receptors : IC_{50} > 100 μM.**

	PDE1	PDE2	PDE3	PDE4	PDE5
IC_{50} (μM)	13	> 200	121	3	36

BWA78U STRUCTURAL OPTIMISATION WITHIN THE ADENINE SERIES
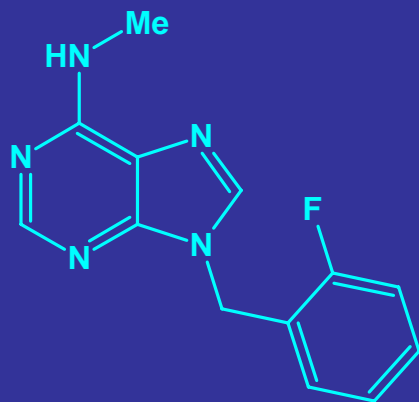
BWA78U

3 μM

NCS 728

1 nM
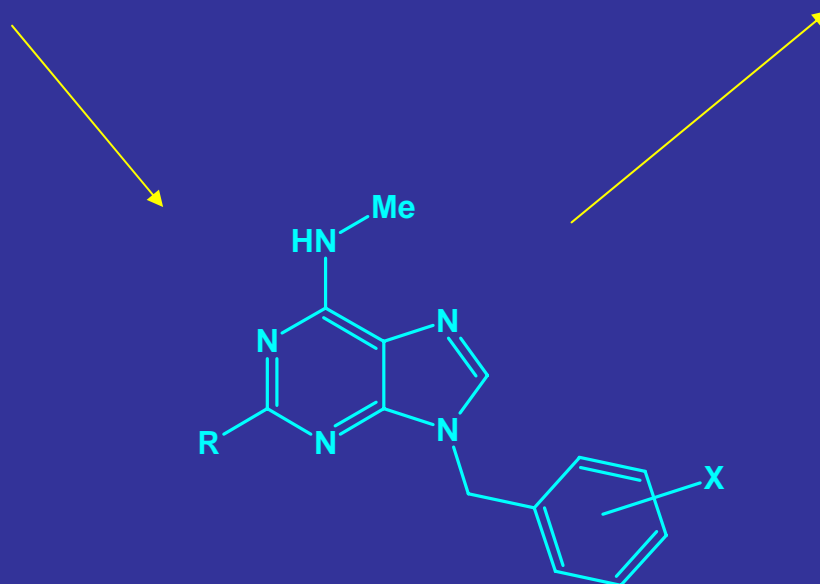
selective towards other PDE isoforms

($IC_{50} > 50$ μM)

ISOSTERES OF ADENINE
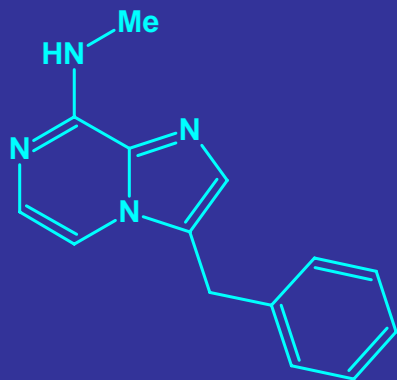
Imidazopyrazines

Adenines

Pyrazolotriazines

optimisation

NCS 823

1 nM

(benzyl series)

FURTHER TOPOLOGICAL EXPLORATION WITHIN THE PYRAZOLOTRIAZINE SERIES

$R = (CH_2)_n$ - functional groups FG

spacer   FG { cations
                      anions
                      H bond A/D
                      hydrophobics
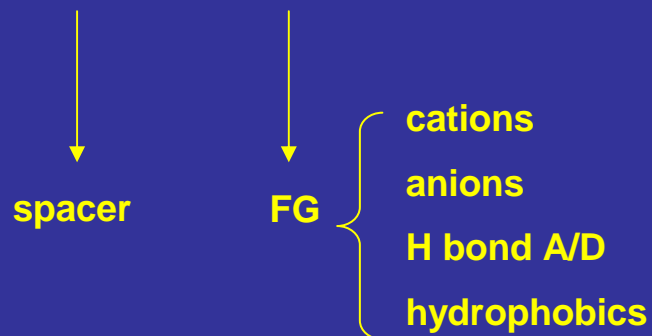
R1	R2	PDE4-I IC50(nM)
Me	$(CH_2)_6$-C(O)NHMe	130
Me	$(CH_2)_2$-C(O)NHOH	30
CF$_3$	$(CH_2)_2$-C(O)NHOH	1,5 *
Me	$(CH_2)_5$-OH	200
Me	$(CH_2)_6$-COOH	300
Me	$(CH_2)_6$-C(O)N(Et)$_2$	90
Me	$(CH_2)_5$-NH-Ph	12

* selective towards other PDE's
(IC50>10 μM)

theophylline
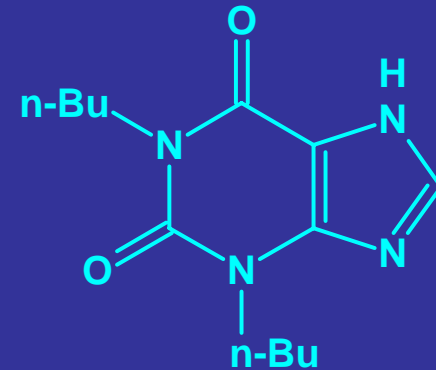
denbufylline

0.8 μM

DESIGN OF PDE4 INHIBITORS

- **Benzodiazepines and related structures**

Parke Davis (0.6 μM)

Warner Lambert (1 μM)

Tofisopam

(0.9 μM)
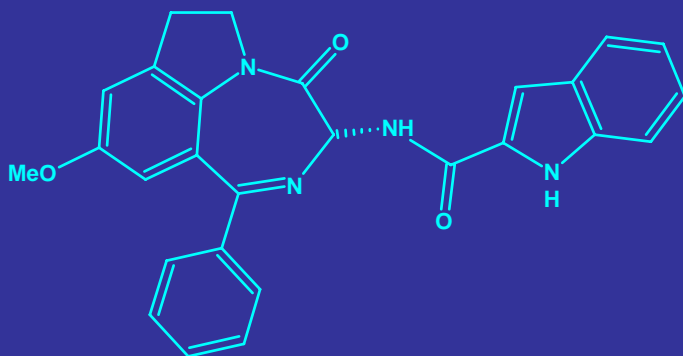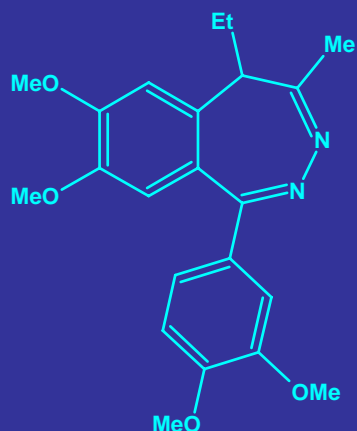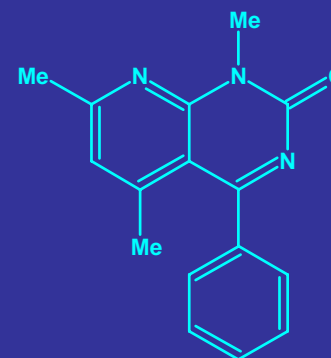
0.22 μM

Gastrointestinal side-effects in ferrets

number of retchings

number of vomits

0.16	0.6	0.04	0.16
roflumilast		ND-716	
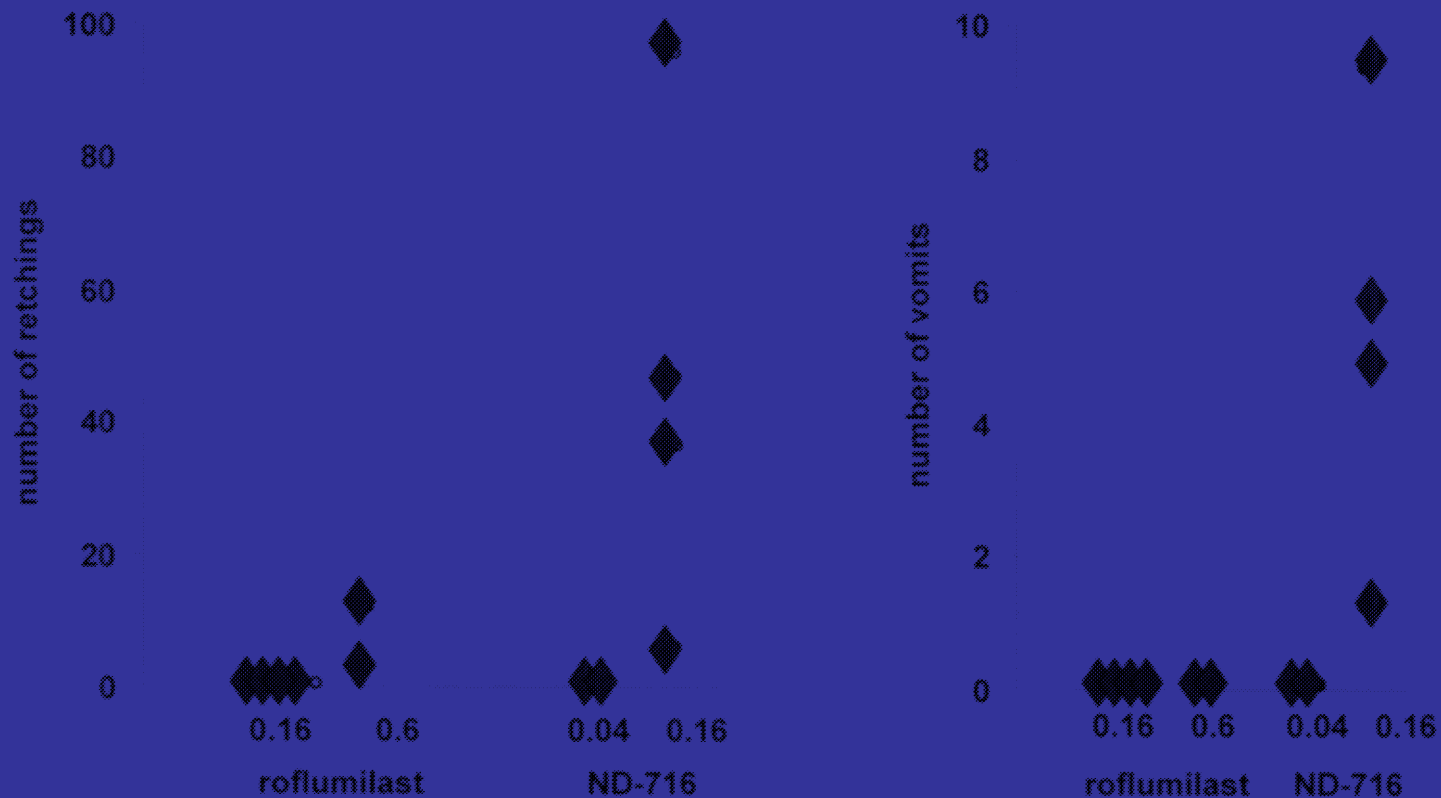
- compounds given orally to fed animals (n = 2-4)
- observation period 4 hour

DEVELOPMENT OF ANTI-INFLAMMATORY COMPOUNDS

Known target

Unknown target

Binding / enzyme inhibition

Cell-based assay

➢ **Receptors :**

- **kinins**
- **prostanoids**
- **neurotrophins**
- **peptides**
- **cytokines**
- **NO**
- **biogenic amines**

➢ **Enzymes :**

- **phosphodiesterases**
- **protein kinases**
- **proteases**
- **Cyclooxygenases**
- **metalloproteinases**
- **NO synthase**
- **phospholipase A2**
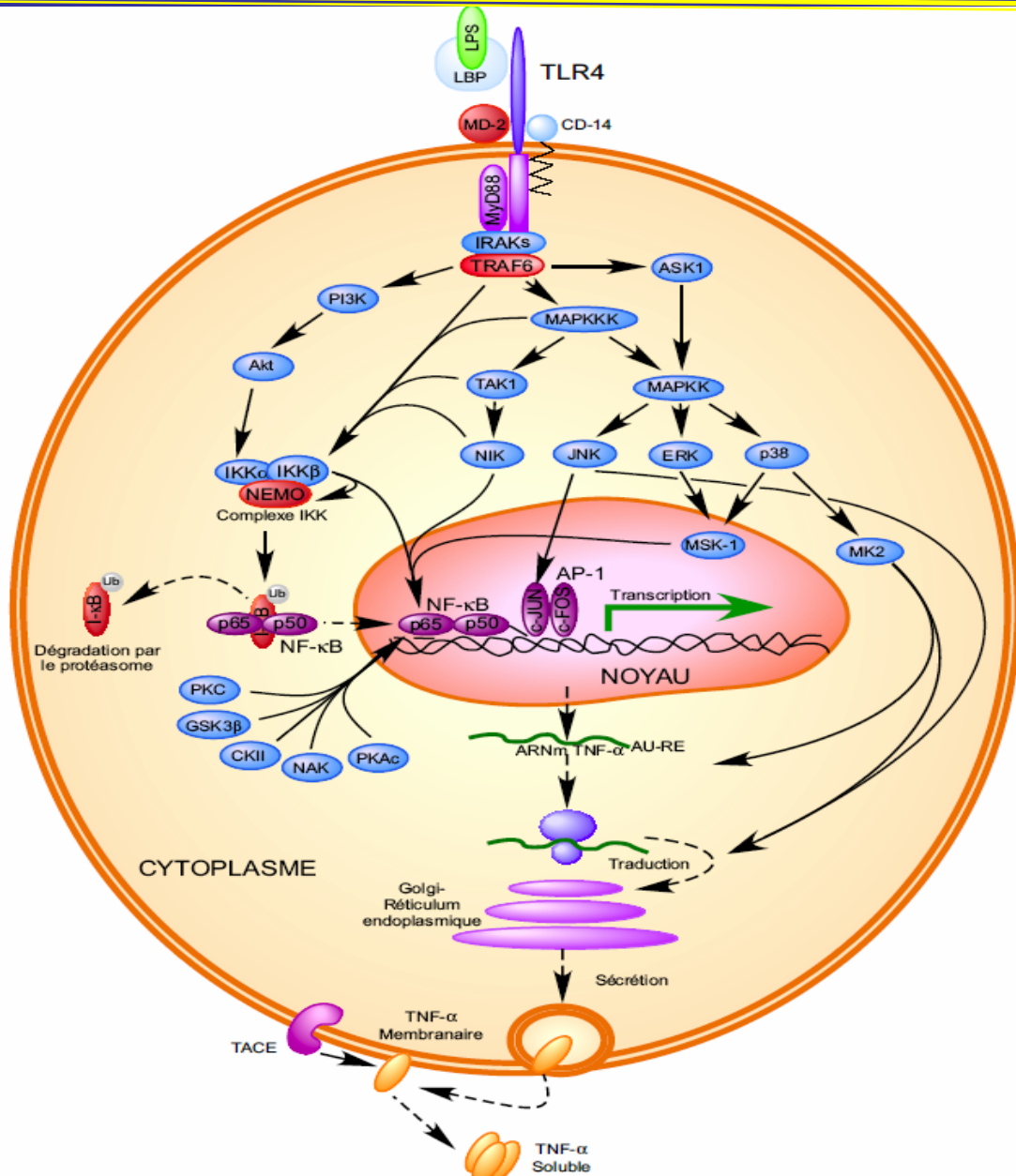
**LPS :
Lipopolysaccharides**

SCREENING OF THE FIRST PART OF THE ULP LIBRARY (1600 compounds)

1600 molécules testées
(chimiothèque n°1)

Exclusion des touches cytotoxiques

Inhibiteurs (≤ 20 % contrôle)

IL-1ß et TNF-α	IL-1ß	TNF-α
17	0	15

32 touches dont 16 possédant une CI$_{50}$ TNF-α < 2µM

in vivo

composé	TNF-α CI$_{50}$ (µM)	IL-1ß
01-02 F10	0,1 ± 0,1	N.A.
01-04H08	0,1 ± 0,1	0,5 ± 0,3
02-02 A05	1,8 ± 0,3	10,1 ± 0,4
02-04 B08	2,0 ± 3,0	N.A

Isolement des cellules mononucléées à partir de sang périphérique humain.

Incubation des cellules mononucléées en présence des composés et de LPS (24h, 37° C).

Surnageants d'incubation

Cellules mononucléées
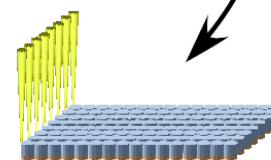
Test ELISA

Test MTT

Dosage du TNF-α et de l'IL-1β

Mise en parallèle des résultats

Viabilité cellulaire

Exclusion des touches cytotoxiques
Composés actifs

IN VIVO VALIDATION

T$_{-30}$: drogue IP (100 mg/Kg)

↓

T$_0$: Injection sous cutanée 100 µl LPS (1mg/ml)

T$_{+2h\ 30}$: anesthésie puis sacrifice et section de la patte centrifugation (1800 g/20min) test Elisa TNF-α

Dosage du TNF-α dans l'exsudat de la patte de rat (n=5)

DEVELOPMENT OF NEW ANTI-INFLAMMATORY DRUGS
CONCLUSIONS AND PERSPECTIVES

Validated Hits

Structural analysis
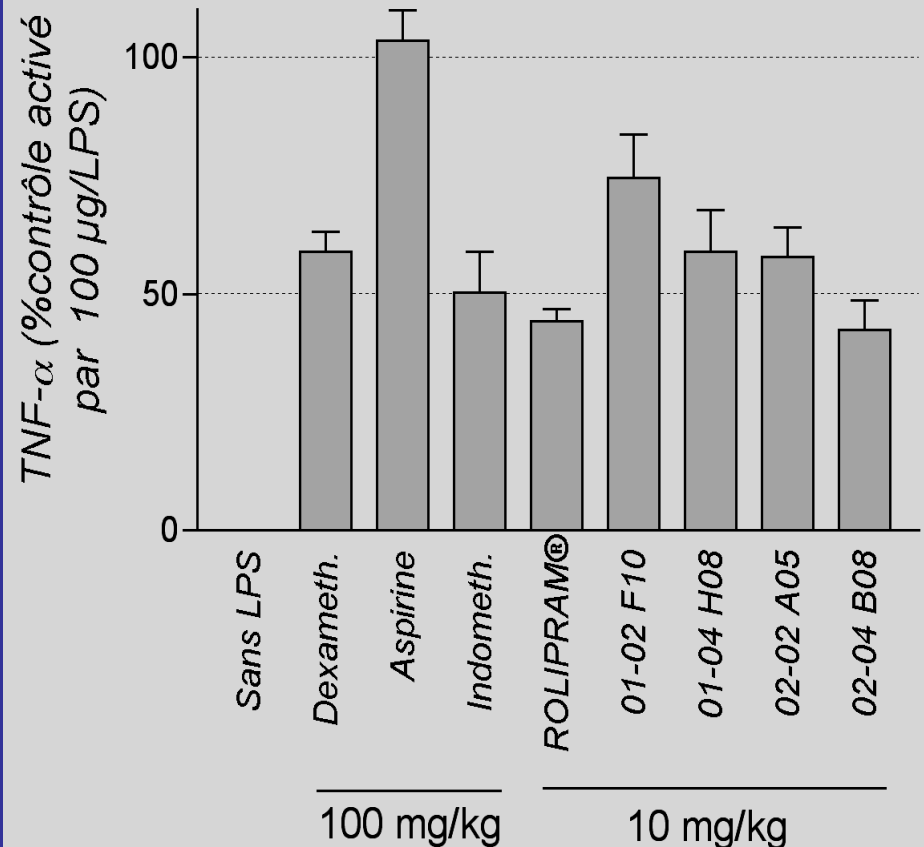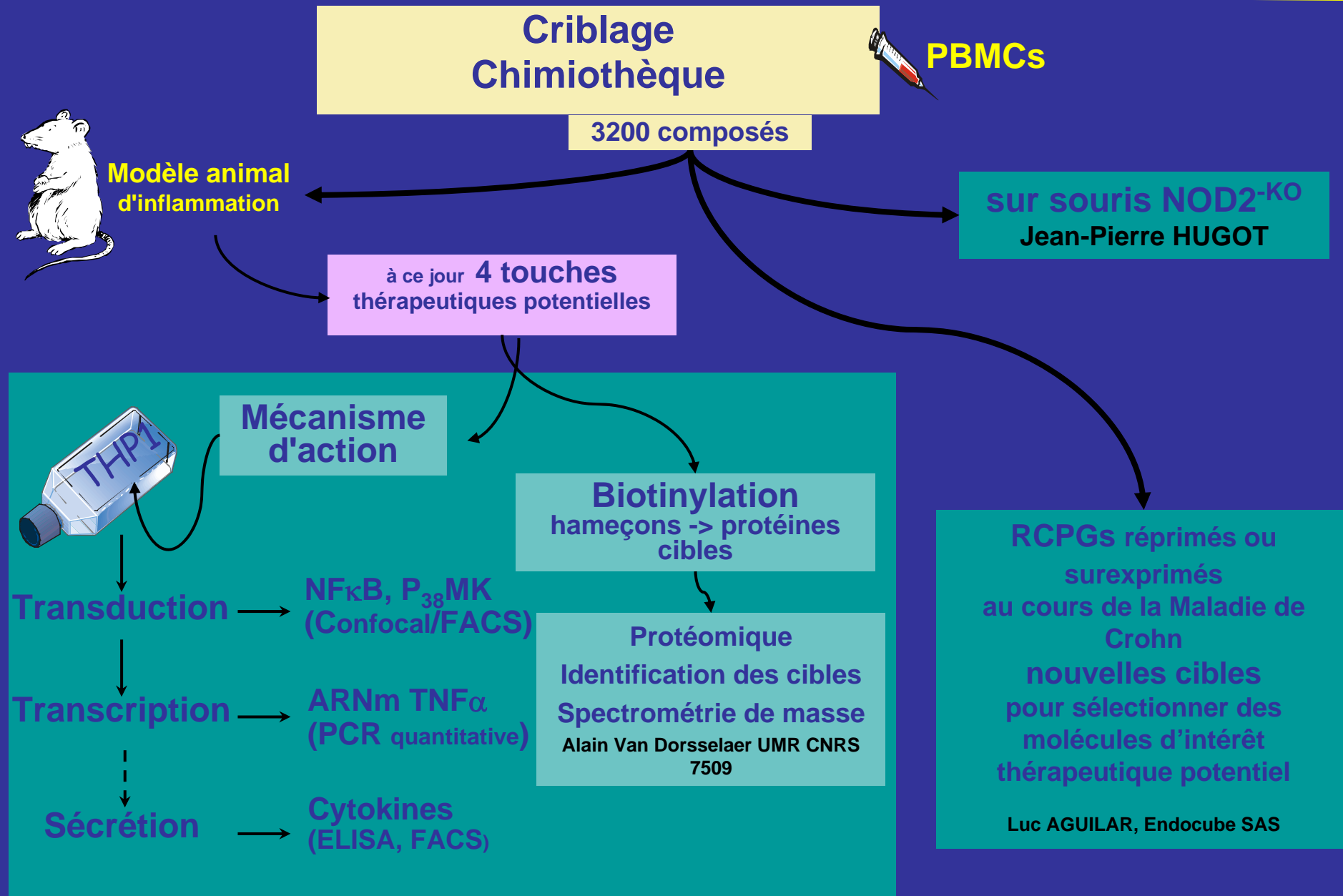Structural clustering, Comparison with literature data, etc.

Pharmacophore analysis

Screening / Cell-based assay (human PBMC's)

Known anti-inflammatories with Unknown mechanism of action
Natural products / ethnopharmacology
In vivo tests
unexpected observations

Classified hits

2nd approach Dealing with Drug optimization

Structural optimization / cell–based assay

In vivo validation / anti-TNF α

New *in vitro* assay / target

Selection of the most promising hits

Chimiogenomics
Isolation and characterization of the target proteins

Further drug optimisation

BUILDING NEW PEPTIDOMIMETICS STARTING FROM SHORT PEPTIDES

❏ RATIONAL APPROACH

Starting from the structure of the endogenous peptidic ligand.

❏ SYSTEMATIC SCREENING

No *a priori* structural hypothesis.

I. Identification of the key aminoacid residues for producing high affinity of binding to the protein target.

OBJECTIVE : small peptides (2-3 aminoacids) with significant affinity (μM range IC_{50}) = "lead compound".

TOOLS : progressive deletion (C- or N-terminal part).
Alanine "scan".
L to D inversion, etc.

II. Structural optimization of the lead compound.

μM range IC_{50} $\longrightarrow$ nM range IC_{50}

Identification of the minimally active fragment along the sequence of amino acids in peptides

| STARTING PEPTIDES	IMPORTANT RESIDUES	DERIVATIVES WITH POTENT AFFINITY
DELTORPHINE (δ OPIOID)	Tyr^1-D-Ala^2-Phe^3	Tyr-D-Ala (μ OR δ AGONISTS)
DERMORPHINE (μ OPIOID)	Tyr^1-D-Ala^2-Phe^3	
FIBRINOGEN	-Arg^{95}-Gly^{96}-Asp^{97}-	RGD MIMETICS
CHOLECYSTOKININ (GASTRIN)	-Trp^5-Asp^7-Phe^8	Trp-Asp PEPTOIDS (ANTAGONISTS)
SUBSTANCE P (NEUROKININS)	-Phe^7-Phe^8-	Phe (NK1, NK2, NK3 ANTAGONISTS)
NEUROPEPTIDE Y	-Arg^{35}-Tyr^{36}	Arg (NPY ANTAGONISTS)

CRITICAL ROLE OF ARGININE IN BINDING OF NPY TO ITS RECEPTOR

H-Tyr[1]-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-
Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-
Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-
Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr[36]-NH$_2$
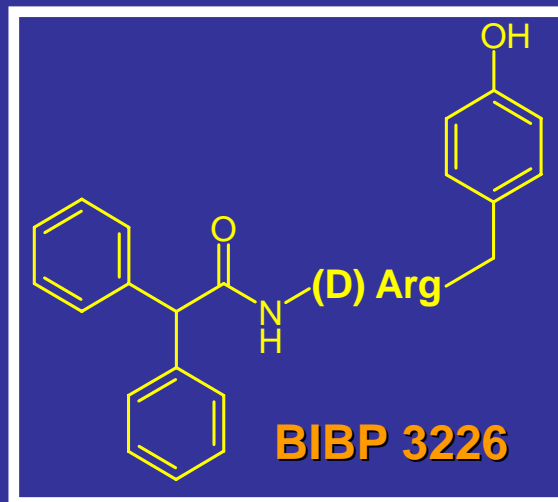
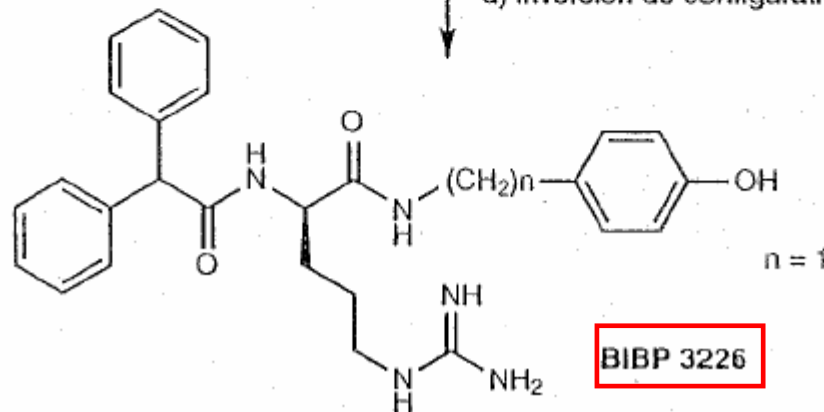
BIBP 3226

BIBP 3226
IC$_{50}$ = 7 nM
NPY1 antagonist
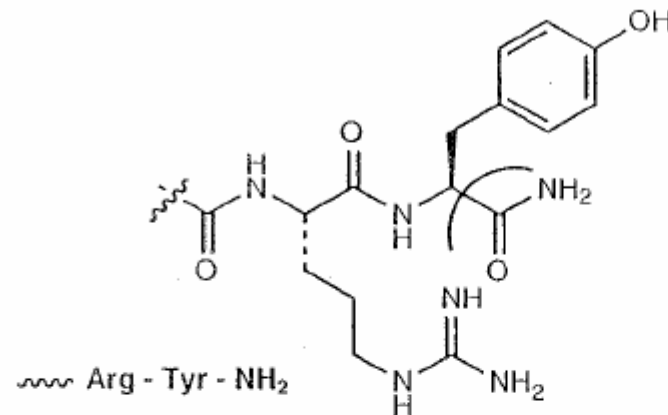
Alanine scan and deletion study of Cam-1666

Cam No.	Structure	NK_2 receptor binding affinity IC_{50} (nM)
1666	Leu-Met-Gln-Trp-Phe-Gly-NH$_2$	14
1701	**Ala**-Met-Gln-Trp-Phe-Gly-NH$_2$	47
1702	Leu-**Ala**-Gln-Trp-Phe-Gly-NH$_2$	160
1727	Leu-Met-**Ala**-Trp-Phe-Gly-NH$_2$	180
1748	Leu-Met-Gln-**Ala**-Phe-Gly-NH$_2$	>10000 ←
1704	Leu-Met-Gln-Trp-**Ala**-Gly-NH$_2$	9600 ←
1705	Leu-Met-Gln-Trp-Phe-**Ala**-NH$_2$	32
1747	Met-Gln-Trp-Phe-Gly-NH$_2$	50
1789	Leu-Met-Gln-Trp-Phe-NH$_2$	230

Conclusions:
Primary importance of Trp and Phe side-chains.

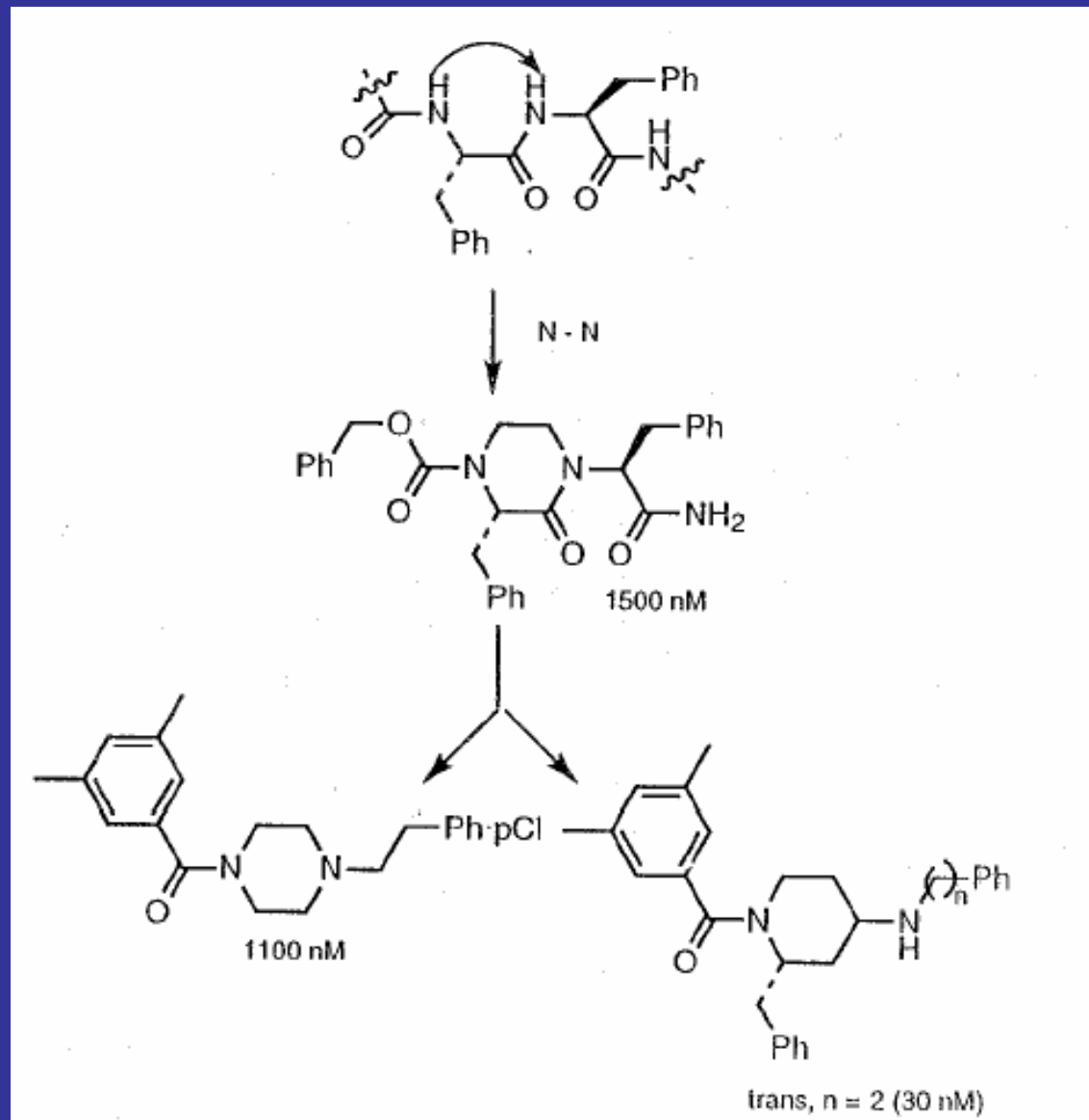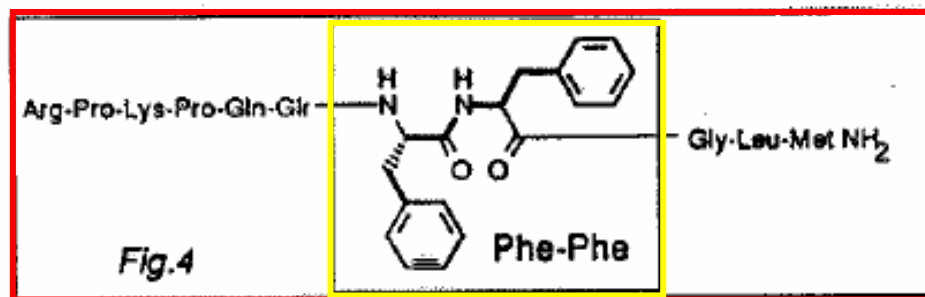Secondary importance of Met and Gln side-chains and Gly amide.

BINDING OF ENDOGENOUS PEPTIDES.
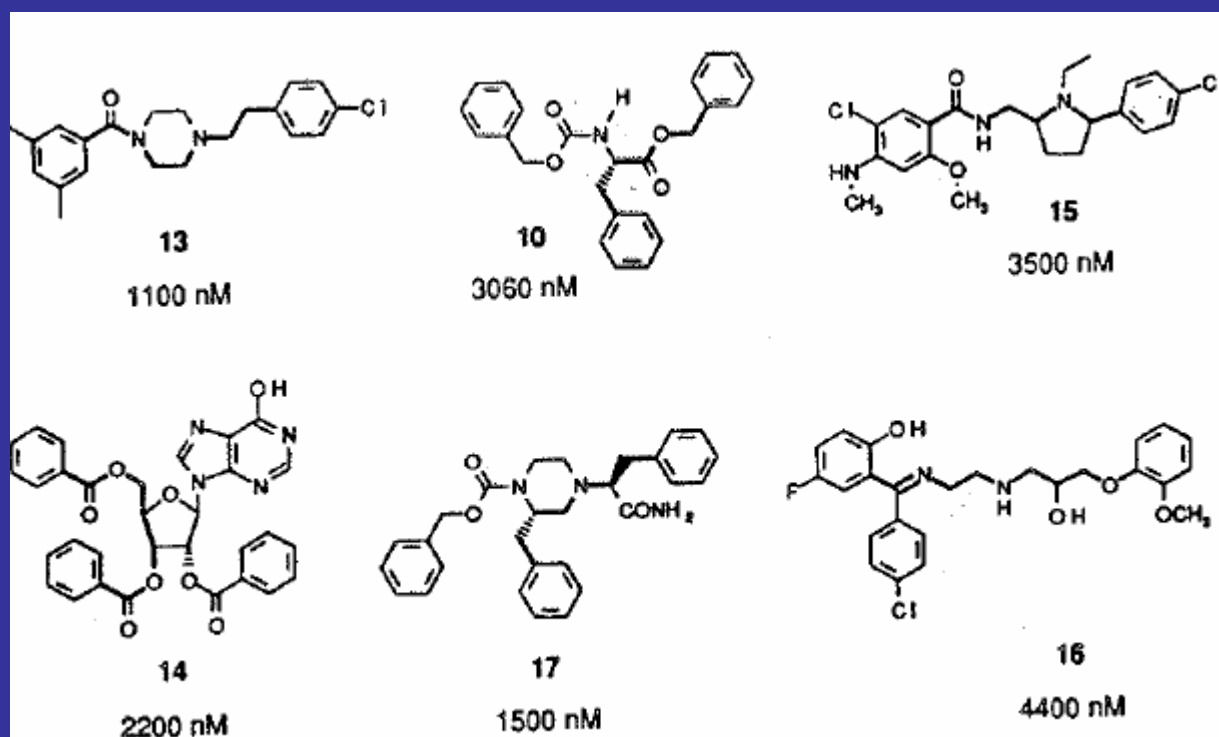# CRITICAL ROLE PLAYED BY PHENYLALANINE

Substance P   Arg-Pro-Lys-Pro-Gln-Gln-**Phe^7-Phe^8**-Gly-Leu-Met-NH$_2$

Cholecystokinine   Asp-Tyr(OSO$_3$H)-Met-Gly-**Trp^5**-Met-**Asp**-**Phe^8**-NH$_2$

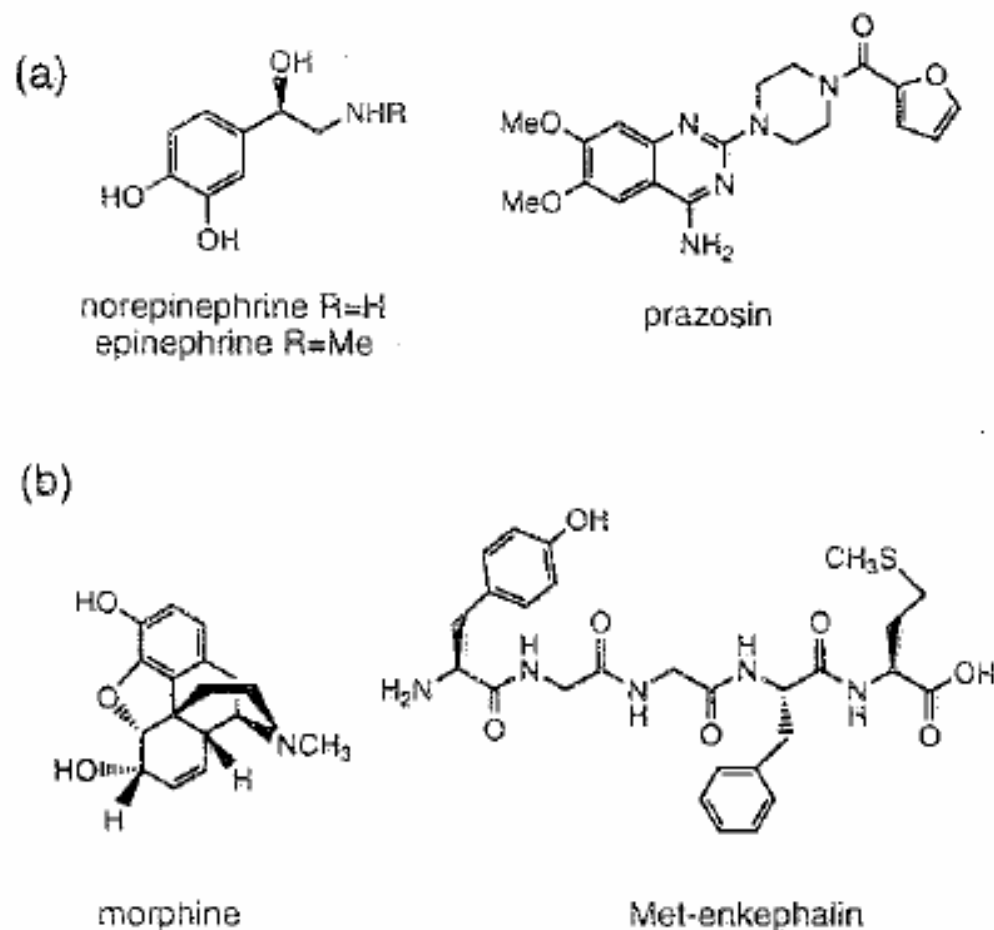**Enkephalines**   Tyr-Gly-Gly-**Phe^4**-Met

Rigidification of Phe-Phe dipeptide

Fig.4

Phe-Phe

Non-peptides and Z-Phe-OBn (10)
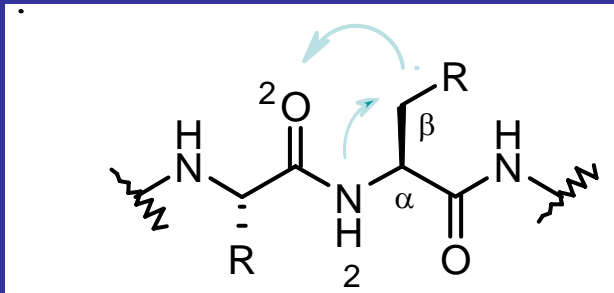**A common structural denominator?**

13
1100 nM

10
3060 nM

15
3500 nM

14
2200 nM

17
1500 nM

16
4400 nM

Figure 6. Known ligands for the (a) α_1-adrenergic and (b) opiate receptors.

Others

Cα-methylation

Reduced

Azapeptides

N-Me substitution

Thiopeptides

Ketomethylene

Incorporation in heterocycles
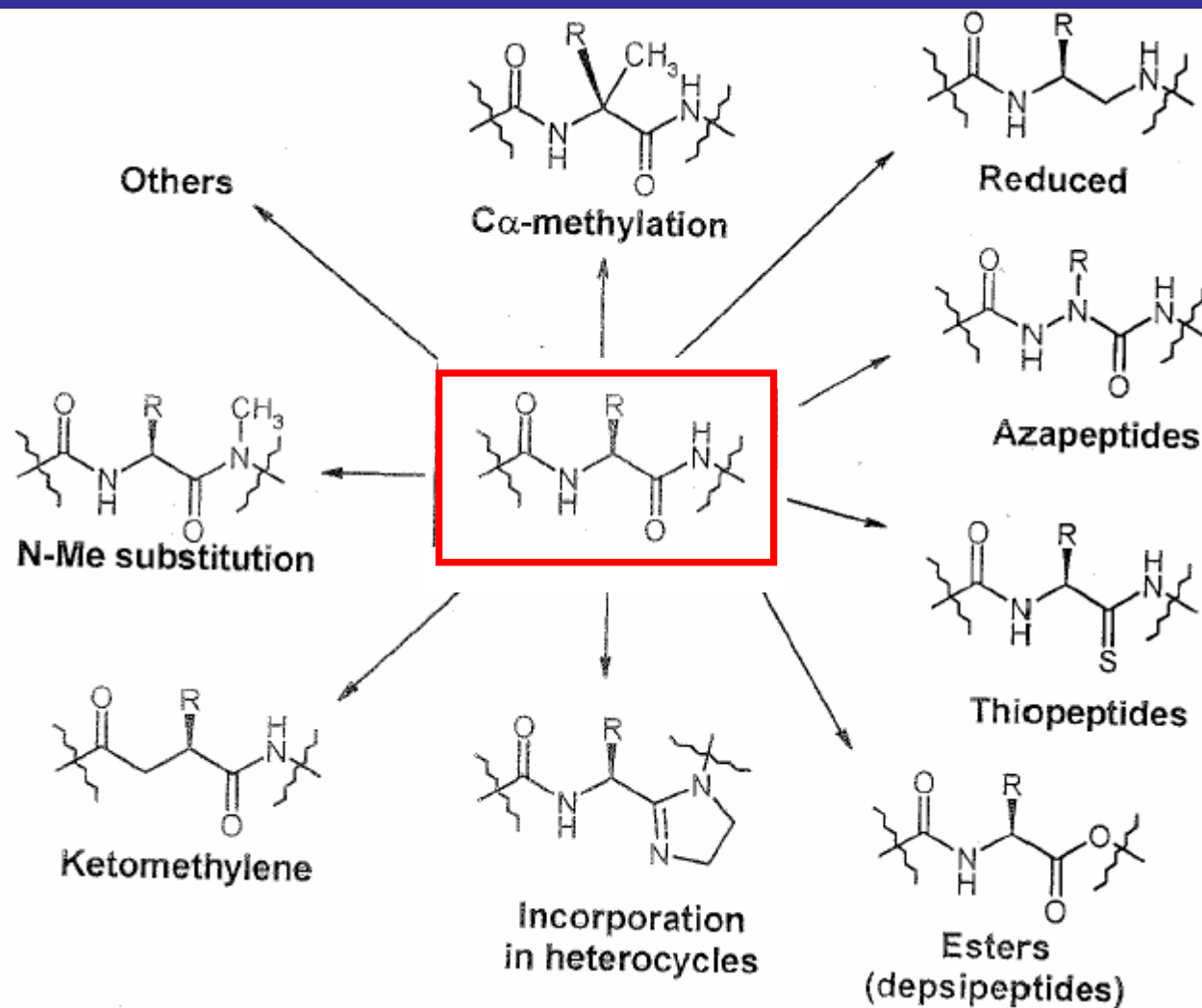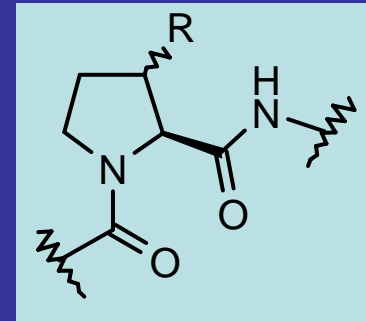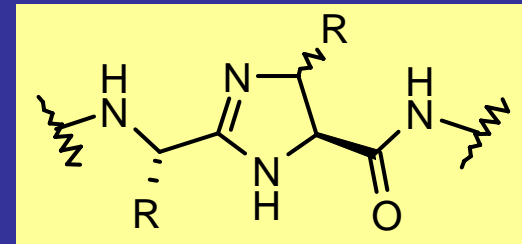
Esters (depsipeptides)
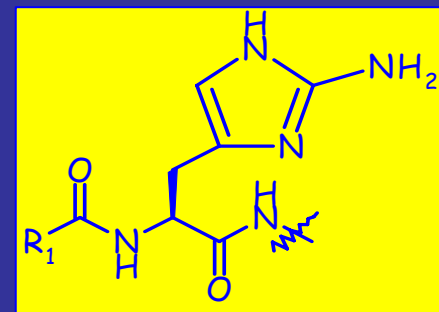
Rigidification de la chaîne latérale de l'arginine
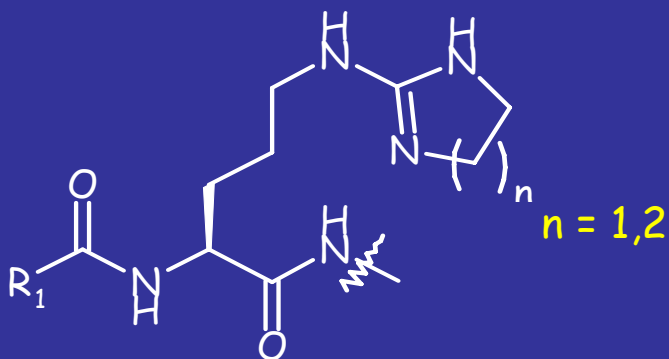
2-amino-imidazolines

$n = 1,2$
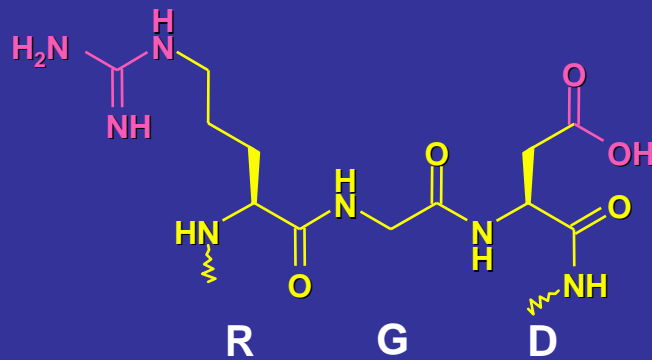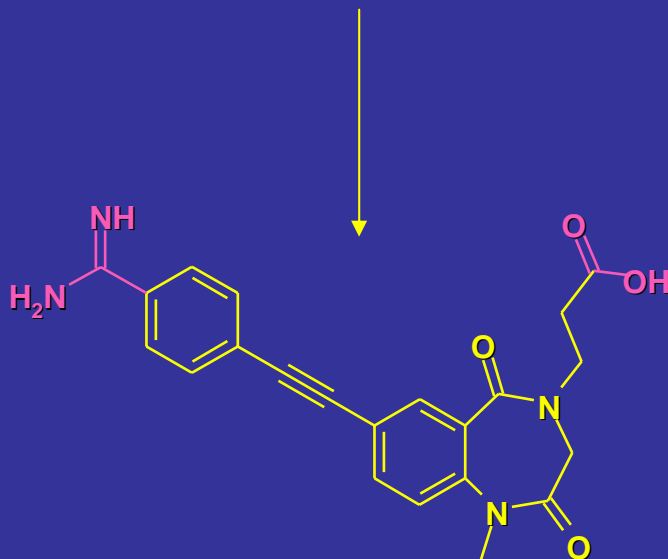
$n = 1,2$
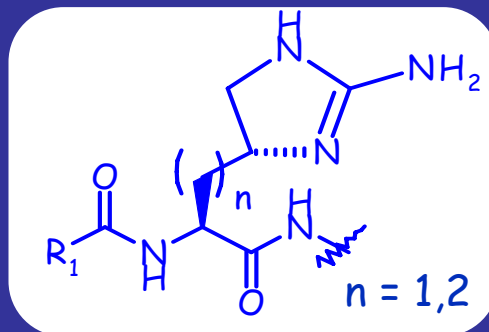
2-amino-imidazoles
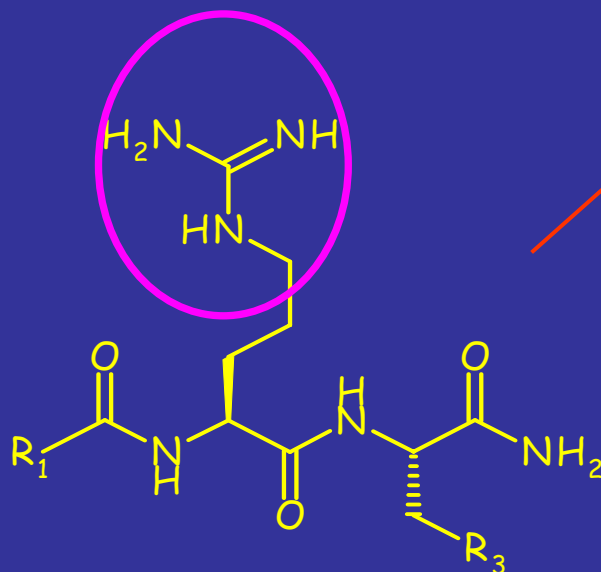
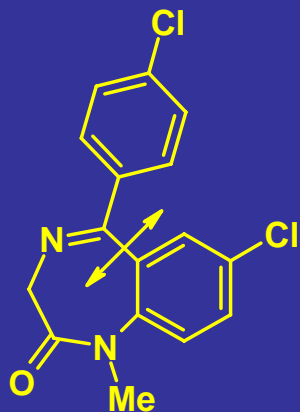**Dipeptide-amide acylé contenant l'arginine**

RGD Mimetics

Ac RGDNH$_2$ CI$_{50}$ 138 μM

CI$_{50}$ 120nM

DÉRIVÉS DE PHÉNYLALANINE COMME LIGANDS DES RÉCEPTEURS MITOCHONDRIAUX AUX BENZODIAZÉPINES

RO 54864
CI_{50} : 3nM
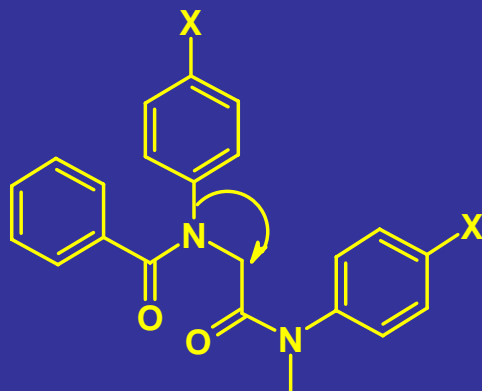
N-phényl-N-acylglycinamide
CI_{50} : 136nM

PEPTOIDE ?

X = H

Dérivés de Phe :

(L), R = Me, inactif
(D), R = H, Me, inactif
(L), R = H, CI_{50} = 28nM

PEPTIDOMIMETIQUE DE DI OU TRI-PEPTIDE

INTERÊTS : - Résistance métabolique accrue.
  - Restriction conformationnelle favorisant les interactions ligand /récepteur.

LE CONCEPT DE PEPTOIDE

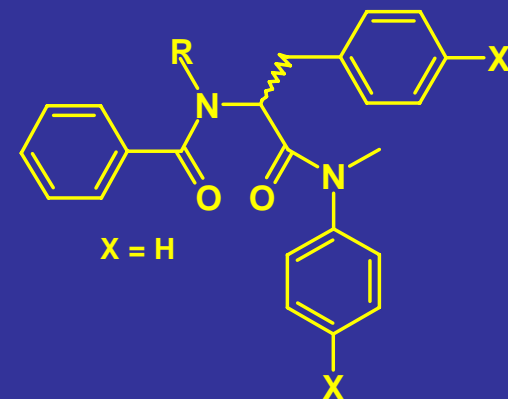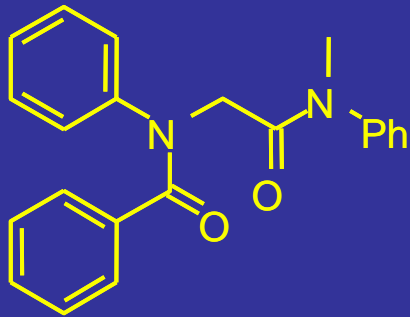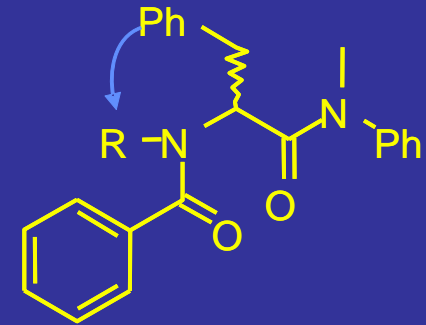**N-phényl-N-benzoyl-glycinamide**

NCS 7027

CI_{50} = 1000 nM

N-benzoyl-phénylalaninamide

R = Me : inactif

R = H : CI_{50} = 28 nM

Derives de Phe comme ligand des R-BZM
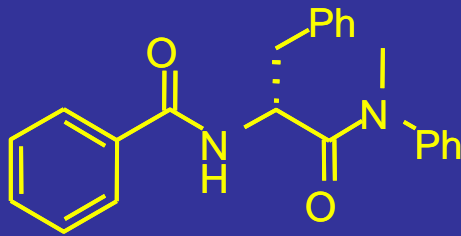
NCS 7084   CI$_{50}$ > 100 nM
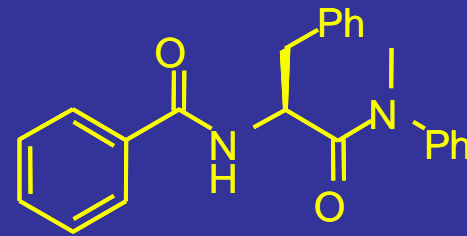
NCS 7083   CI$_{50}$ = 7 nM

Diazépam Binding Inhibitor (DBI) $\longrightarrow$ **Triakontatétraneuropeptide (TTN)**

N-terminal
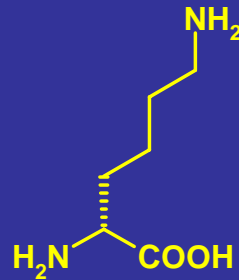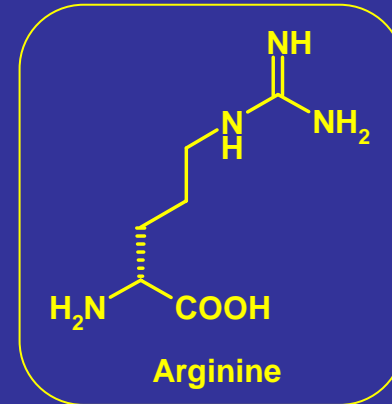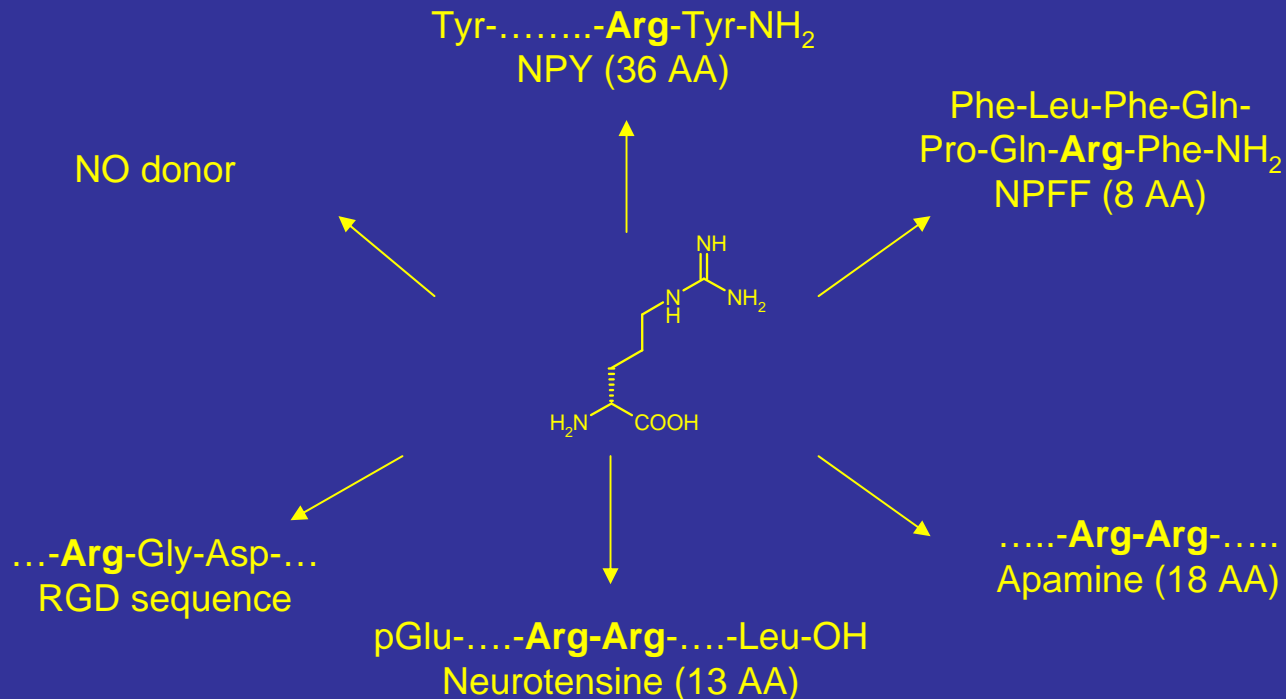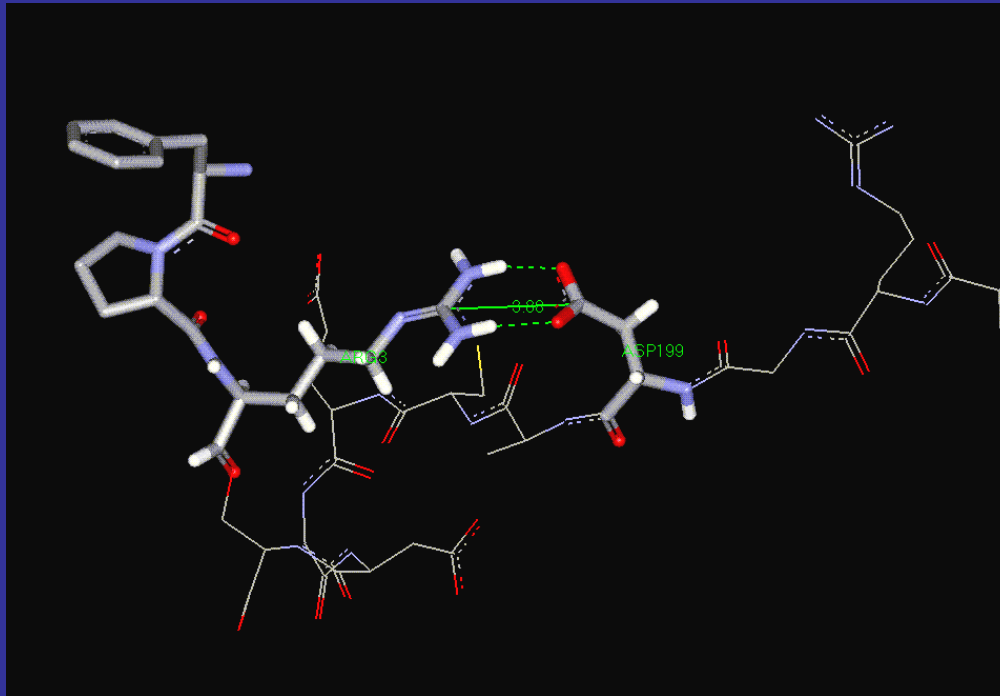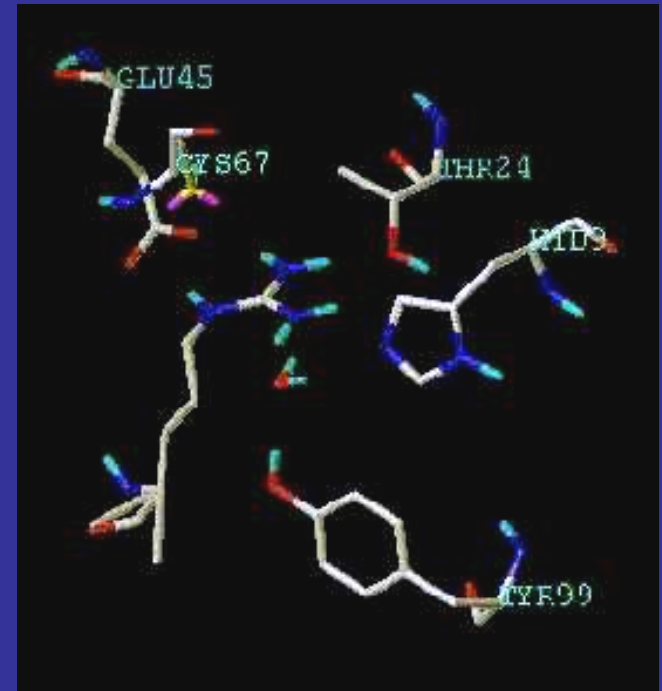
~~~~~~Thr^{17}-Gln-Pro-Thr^{20}-Asp-Glu-Glu^{23}-Met-Leu-**Phe**-Ile-Tyr^{28}-Ser-His^{30}-**Phe**-Lys-Gln-Ala-Thr-Val-Gly-Asp-Val-Asn^{40}-Thr-Asp-Arg^{43}-Pro-Gly-Leu-Leu-Asp-Leu-Lys^{50}~~~~~~

C-terminal

Development of NPFF receptor antagonists

GENERAL POINTS CONCERNING ARGININE

☞ **Basic amino-acids** :

Histidine Lysine Ornithine Arginine

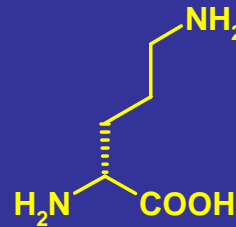☞ **Implicated in a large number of biological effects** :

Tyr-……..-**Arg**-Tyr-NH$_2$
NPY (36 AA)

Phe-Leu-Phe-Gln-
Pro-Gln-**Arg**-Phe-NH$_2$
NPFF (8 AA)

NO donor

…-**Arg**-Gly-Asp-…
RGD sequence

pGlu-…..-**Arg-Arg**-….-Leu-OH
Neurotensine (13 AA)

…..-**Arg-Arg**-…..
Apamine (18 AA)

ARGININE : TOWARD COMPLEX INTERACTIONS

☞ Four types of interactions reported in the PDB :

- electrostatic :

- Hydrogen bond acceptor or donor :

Interaction between Arg of D-Phe-Pro-Arg
chloromethylketone (PPACK) and Asp. 199
of α-thrombine (1HAI)

HLA-B27 site interacting with
Arg 2 of a nonapeptide

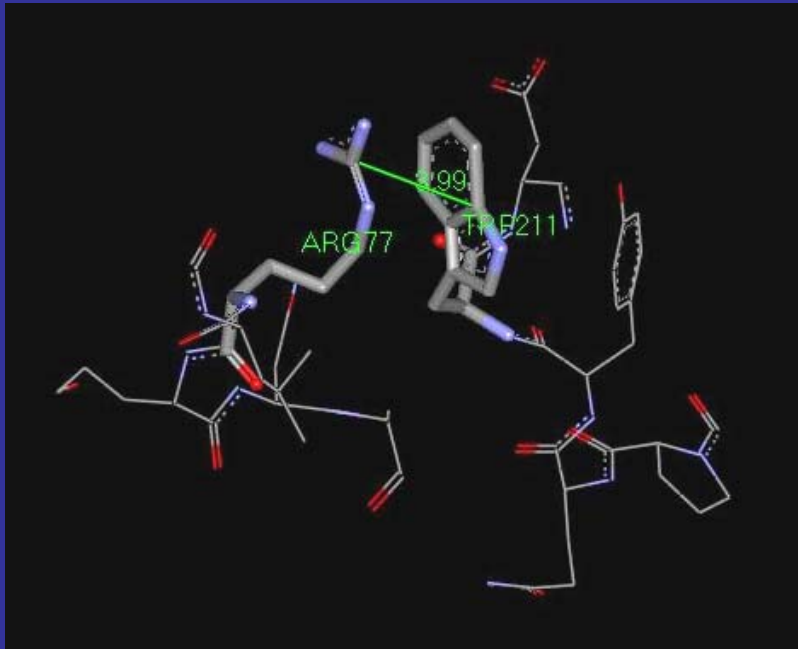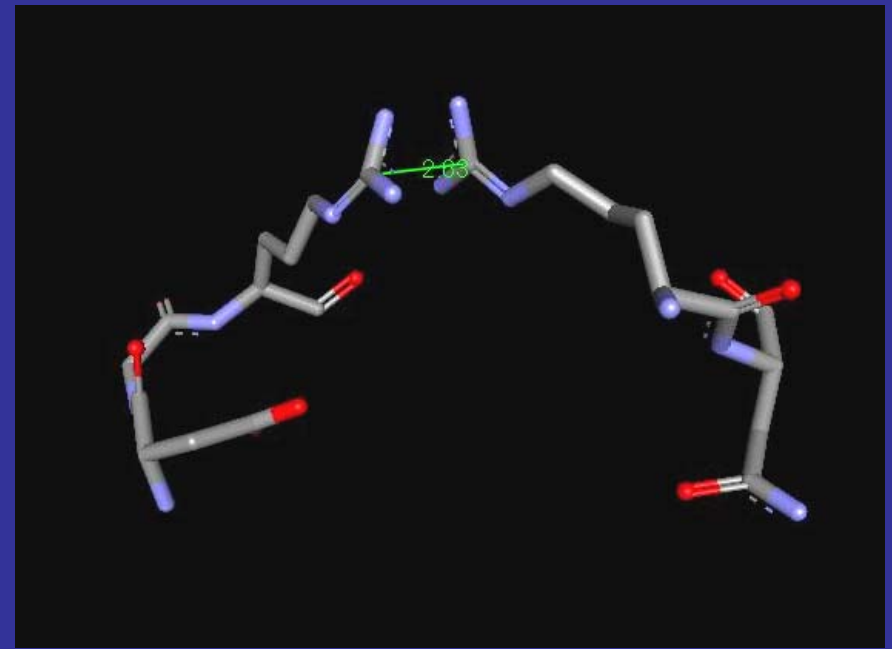# ARGININE : TOWARD COMPLEX INTERACTIONS

☞ Four types of interactions reported in the PDB :

- π-cation :

- Cation-cation :

Interaction between the guanidine function of Arg 77A
and indole ring of Trp 211A of OPPA
(oligopeptide binding protein) (1JET)

Interaction between the guanidine function of Arg 45
and the guanidine function of Arg 68
in a lysozyme (8LYZ)

Le Neuropeptide FF

un octapeptide endogène

Phe-Leu-Phe-Gln-Pro-Gln-Arg-PheNH$_2$

libération suite à la stimulation des récepteurs opioïdes
liaison à des récepteurs propres
effets opposés aux effets analgésiques des opioïdes

→ **Système anti-opioïde endogène**

Applications potentielles des ligands des récepteurs du NPFF

- Traitement de la douleur

- Traitement de la toxicomanie opioïde

Identification des sites

NPFF   Phe Leu Phe Gln Pro Glu Arg Phe NH

^{125}I NPFF ^{125} Tyr Leu Phe Gln Pro Glu Arg Phe NH

K_D = 0.1 nM

ligands opiacés, substance P, CCK, NPY, MIF1 : pas de liaison à 10 M

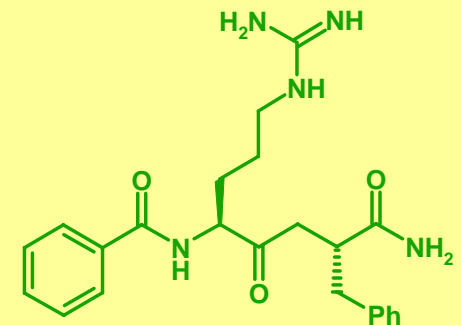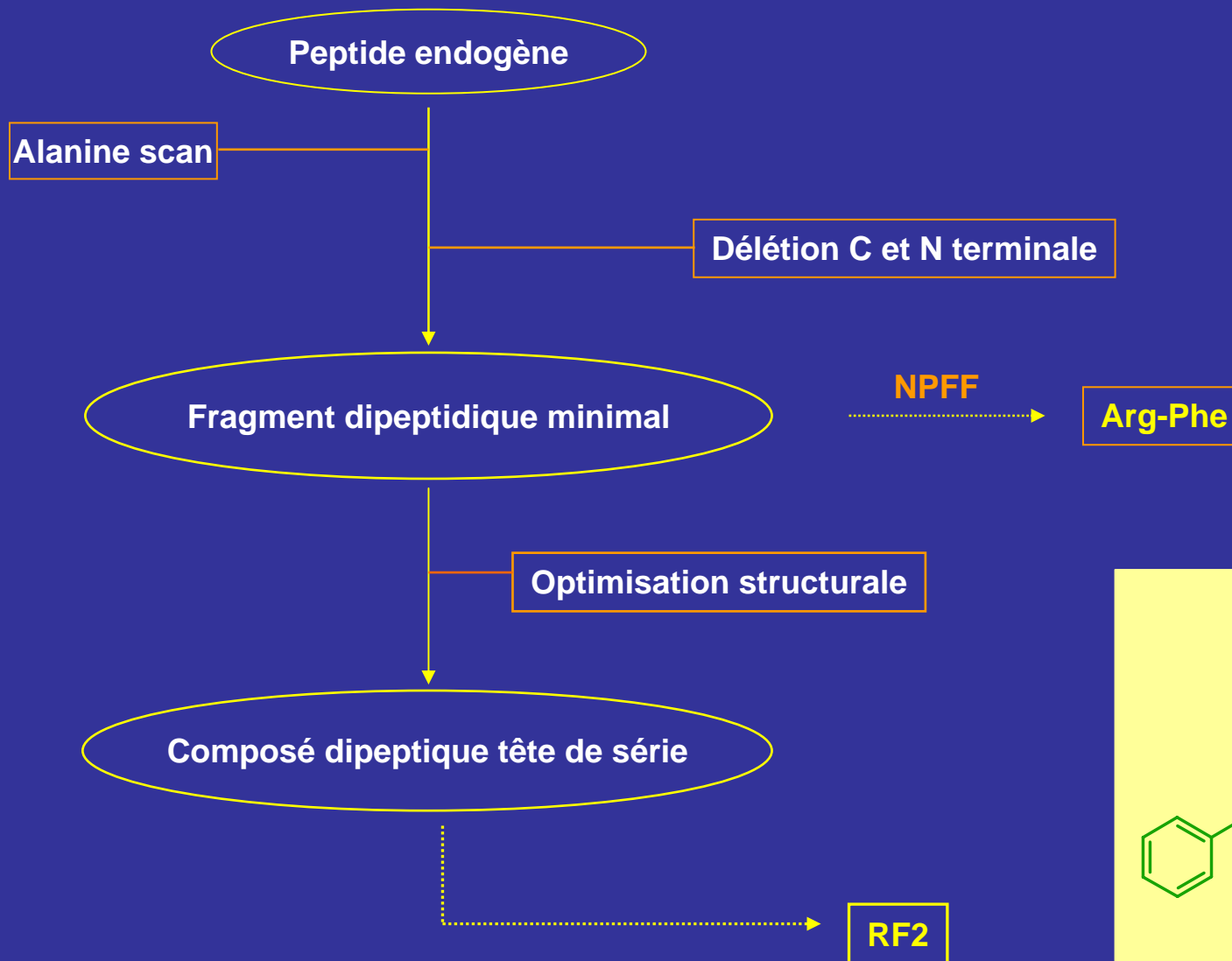affinité du NPFF pour les récepteurs aux opiacés : Ki   = 5 M

Ki   >10 μM

Distribution : moelle épinière, substance grise péri aqueducale, hypothalamus, structures dopaminergiques, lymphocytes, coeur

Couplage protéine G : second messager ?

Importance du tétrapeptide C -terminal du NPFF pour la liaison au récepteur

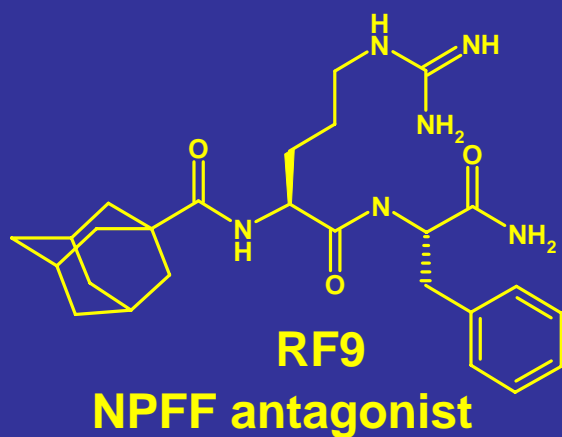|										Ki nM
NPFF	Phe	Leu	Phe	Gln	Pro	Gln	Arg	Phe	NH$_2$	0,25
	Phe	Leu	Phe	Gln	Pro	Gln	Arg	Phe	OH	900
PQRF amide					Pro	Gln	Arg	Phe	NH$_2$	12
FMRF amide						Phe	Met	Arg	Phe	NH$_2$	1,8
FFRF amide						Phe	Phe	Arg	Phe	NH$_2$	0,25

NPFF Project

NPFF: Phe-Leu-Phe-Gln-Pro-Gln-Arg-PheNH$_2$ IC$_{50}$= 0.1nM

RF9
NPFF antagonist

IC$_{50}$= 75nM

Some semi-rigid derivatives

Propriétés pharmacologiques de RF2

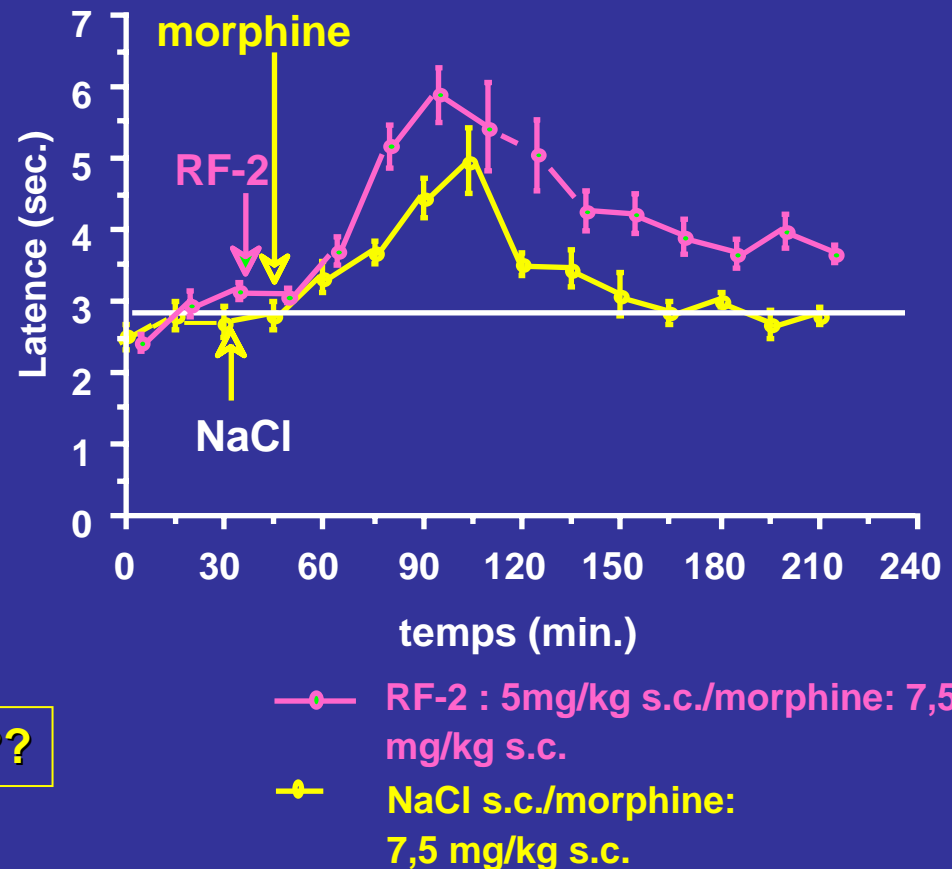**RF2**

. K_i ~ 7nM sur NPFF natif

. Ki ~ 500nM sur NPFF2 (humain)

. Pas d'effet analgésique intrinsèque

. Potentialise les effets analgésiques de la morphine

RF-2 : 5mg/kg s.c./morphine: 7,5 mg/kg s.c.

NaCl s.c./morphine: 7,5 mg/kg s.c.

RF2 antagoniste récepteur NPFF ??

Test fonctionnel : $[^{35}S]GTP\gamma S$

RF2 = antagoniste

Two different neuropeptide FF receptor subtypes and two different peptide ligands

```
hNPAF                          AGEGLSSPFWSLAAPQRF-NH2
hNPFF                          SQAFLFQPQRF-NH2
hNPSF      SLNFEELKDWGPKNVIKMSTPAVNKMPHSFANLPLRF-NH2
hNPVF                          VPNLPQRF-NH2
```

NPFF-1 R   <->   NPSF and NPVF

NPFF-2 R   <->   NPFF and NPAF

Cloning of NPFF receptors

- Nabil et al., 2000 JBC 275, 25965-71: NPFF2 receptor
- Hinuma et al., 2000 Nature Cell Biol 2, 703-708: NPFF1 receptor
- Bonini et al., 2000 JBC 275, 39324-31: NPFF1 and NPFF2 receptors

Cloning of a gene encoding two novel neuropeptides

- Hinuma et al., 2000 Nature Cell Biol 2, 703-708
- Liu et al., 2001 JBC 276, 36961-69

NPFF2 receptor is most likely involved in the control of nociception.

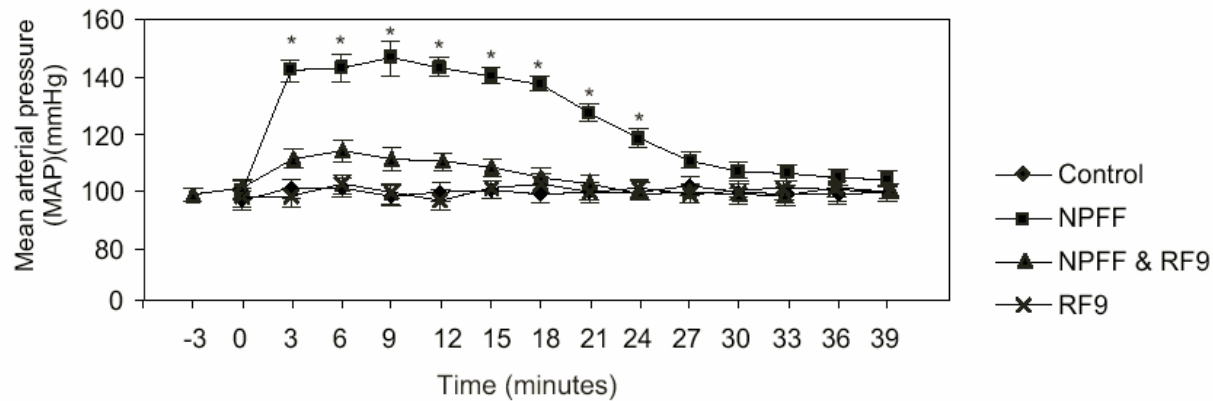## NPFF1 and NPFF2 receptor mRNAs in rats

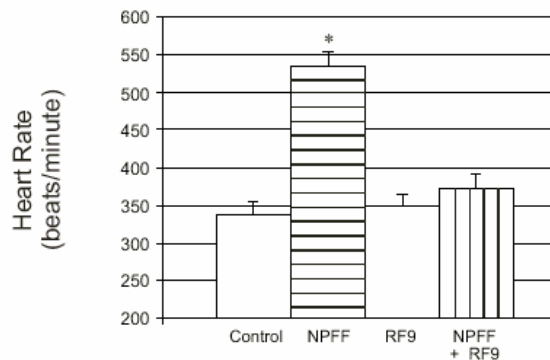| Tissue	rNPFF1	rNPFF2
Adipose	3	12
Adrenal cortex	3	5
Adrenal medulla	17	Trace
Amygdala	57	42
Aorta	1	24
Celiac plexus	4	12
Cerebellum	17	10
Cerebral cortex	22	11
Choroid plexus	25	30
Colon	Trace	8
Dorsal root ganglia	3	38
Duodenum	Trace	5
Heart	3	82
Hippocampus	20	8
Hypothalamus	100	84
Kidney	1	20
Liver	2	3
Lung	4	16
Medulla	22	92
Nucleus accumbens	35	11
Olfactory bulb	41	10
Ovary	14	12
Pancreas	Trace	Trace
Pineal	Trace	4
Pituitary	24	34
Retina	14	40
Salivary gland	Trace	33
Spinal cord	24	100
Spleen	Trace	Trace
Stomach	Trace	14
Skeletal muscle	Trace	Trace
Striatum	17	16
Substantia nigra	49	67
Testes	43	4
Thalamus	3	15
Thymus	Trace	12
Trigeminal ganglia	16	57
Urinary bladder	Trace	16
Uterus	Trace	Trace
Vas deferens	Trace	Trace
Whole brain	21	24

From Bonini et al., 2000

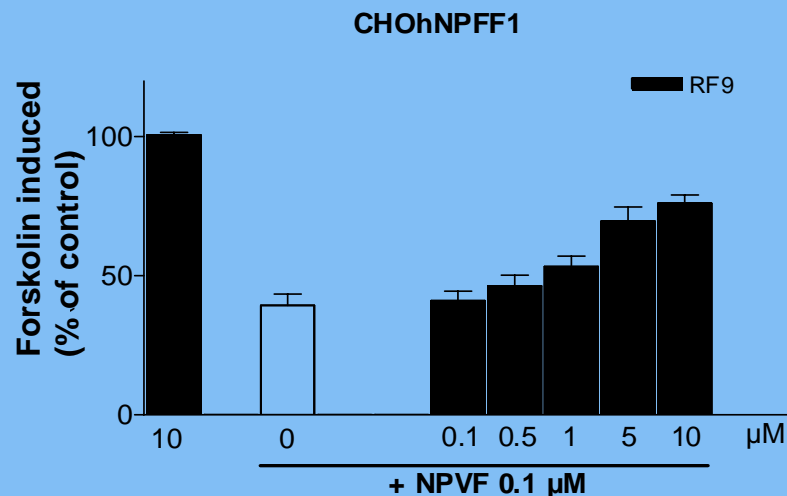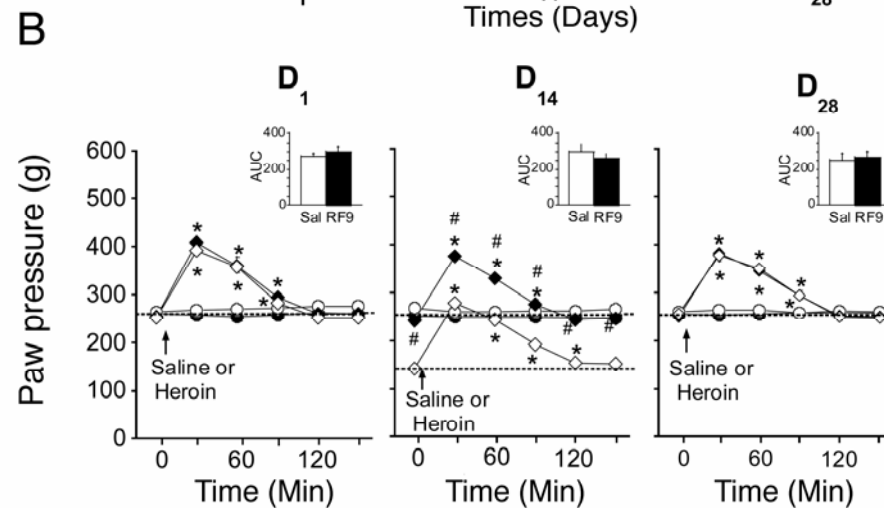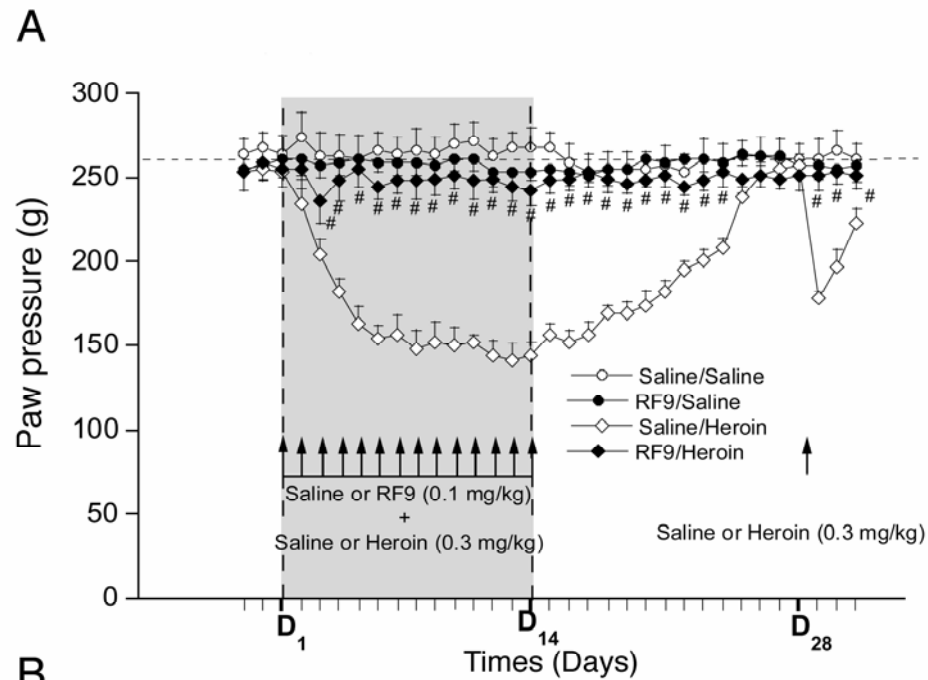# RF9 prevents blood pressure effects elicited by NPFF *in vivo.*

Binding affinities for hNPFF1 receptor of peptides selected from the screening and effect on intracellular cAMP production induced by forskolin).
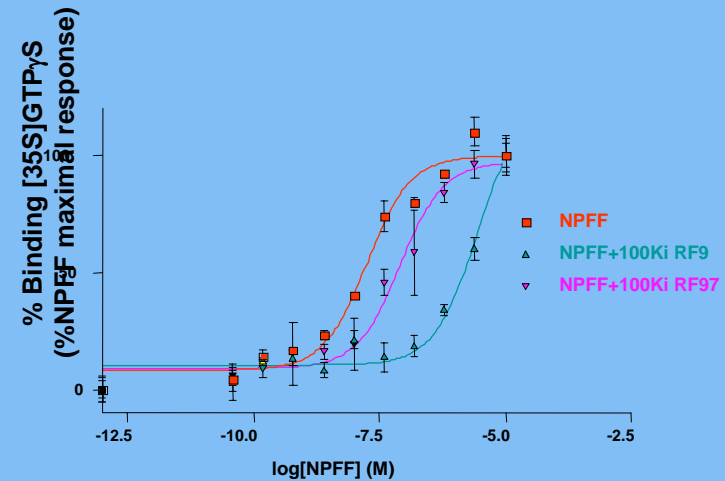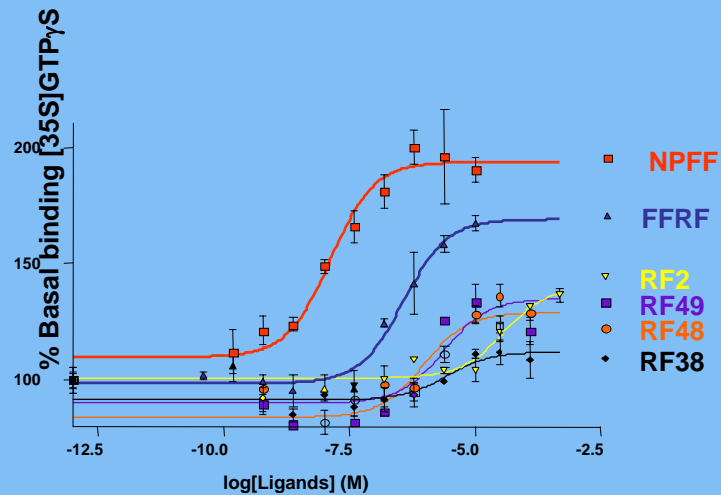
compound	Binding Ki, nM	AMPc EC50, nM	IC50 antagoniste, nM
RF2	756 ± 91 (4)	> 10 000	
RF9	58 ± 5 (4)	> 10 000	4773 ± 1231 (3)
RF38	1233 ± 118 (3)	> 10 000	
RF48	169 ± 11 (4)*	> 10 000	
RF49	153 ± 6 (4)	> 10 000	
RF97	696 ± 42 (4)	> 10 000	
FFRF	14 ± 2 (5)		
bNPFF	9.8 ± 0.8 (3)		
NPVF (VPNLPQRFa)	1 ± 0.2 (3)		

CHOhNPFF1

RF9

Forskolin induced (% of control)

100

50

0

10   0   0.1 0.5 1   5   10   µM

+ NPVF 0.1 µM

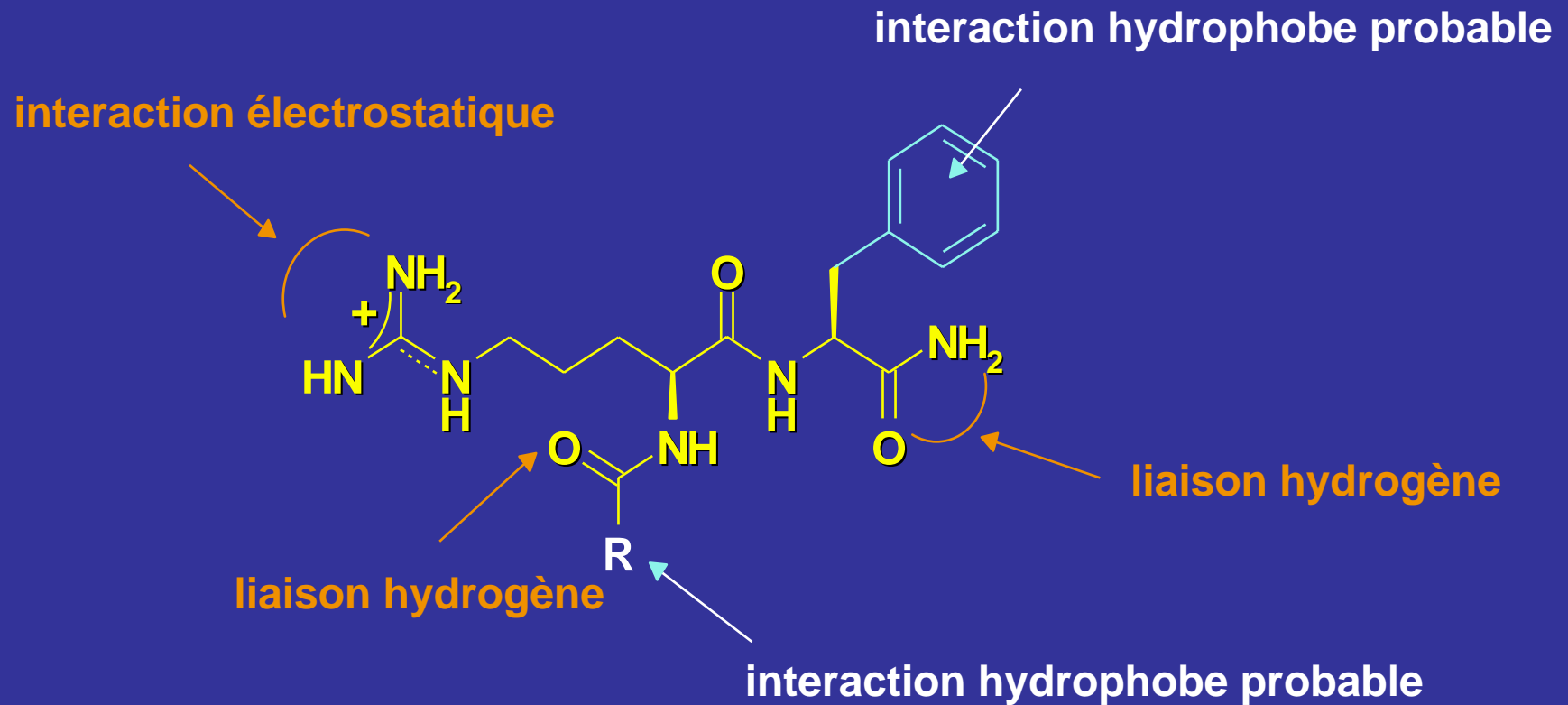# RF9 completely blocks heroin-induced hyperalgesia and associated tolerance.

Stimulation of [35S]GTPγS binding by NPFF and peptides selected from the screening

Ligands	EC_{50} (nM)	Maximal Activation (%)
NPFF	24 ± 4.2	202 ± 8.5
RF2	> 10 000	133 ± 7
RF9	> 100 000	-
RF38	> 1000	125 ± 13
RF48	> 1000	145 ± 10.5
RF49	694 ± 103	122 ± 6
RF97	> 100 000	-
FFRF	567 ± 181	175 ± 5

RF9 (7.5 µM) and RF97 (27 µM) shifted the concentration effect curve of NPFF to the right by about 160-fold and 5 fold respectively

Premier modèle de pharmacophore pour les ligands du récepteur du NPFF

interaction hydrophobe probable

interaction électrostatique

liaison hydrogène

liaison hydrogène

interaction hydrophobe probable

Du Peptide au Peptidomimétique

RF2 : Composé dipeptidique tête de série

• Mauvaise biodisponibilité
• Faible stabilité métabolique

Optimisation en série peptidique

Remplacement des liaisons amides

Pharmacophore

Suppression du caractère peptidique
Génération d'α-aminoacides non-naturels

Restriction conformationnelle
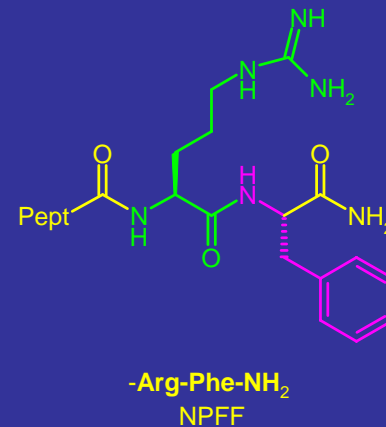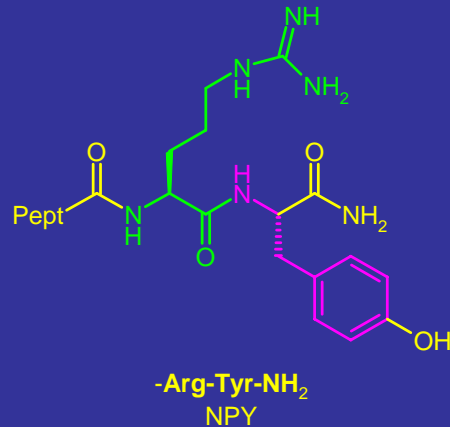
Peptidomimétiques

Applications thérapeutiques

RESEARCH HYPOTHESIS

☞ **Analogy between C-terminal parts of NPFF and NPY :**

-Arg-Tyr-NH$_2$
NPY

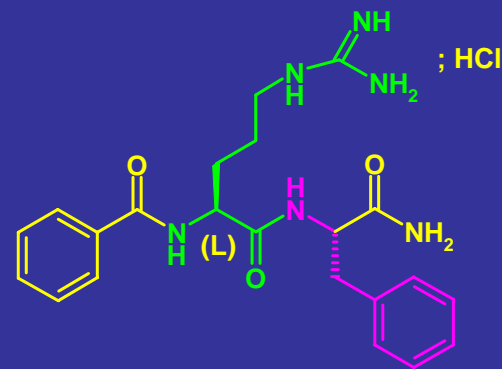**-Arg-Phe-NH$_2$**
NPFF

$\Rightarrow$ Presence of an arginine residue both in NPY and NPFF
$\Rightarrow$ Presence of aromatic amino-acids (Phe et Tyr)
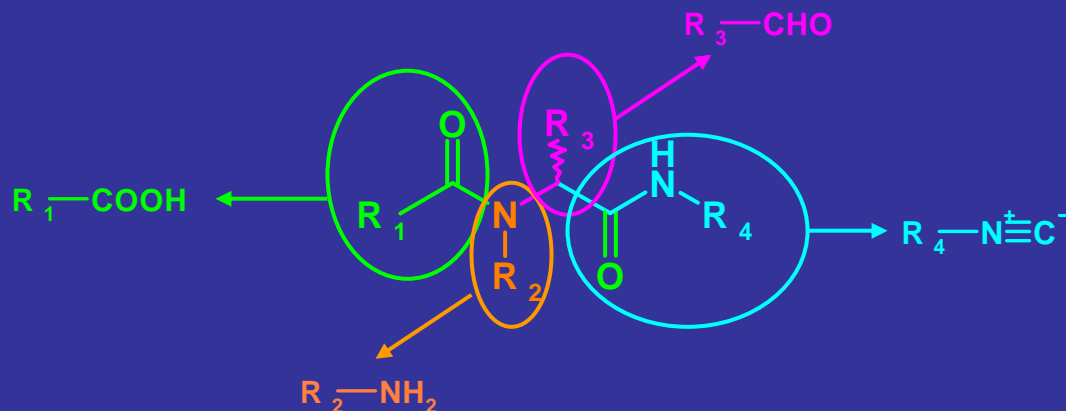
☞ **Analogy between 2 ligands NPFF/NPY :**

BIBP 3226

$IC_{50} = 17$ nM (Y_1)
$K_i = 25$ nM (hNPFF-1)
$K_i = 1\,585$ nM (hNPFF-2)

RF-2

inactive on Y_1
$K_i = 756$ nM (hNPFF-1)
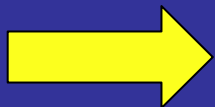$K_i = 526$ nM (hNPFF-2)

GENERAL POINTS CONCERNING UGI REACTION

☛ Use of four components : aldehyde, amine, carboxylic acid,isocyanide.

☛ Formation of racemic peptides.

☛ Easy introduction of molecular diversity.

☛ Rapid access to peptides.

☛ Satisfactory global yields.

CONCLUSIONS

- RF9 is a potent and selective antagonist for NPFF receptors both in vitro and in vivo.

- RF9 can prevents opioid-induced hyperalgesia and associated tolerance.

➡️ NPFF system is a *bona fide* anti-opioid system.

NPFF antagonists could represent useful therapeutic agents for improving the efficacy of opioids in chronic pain treatments.