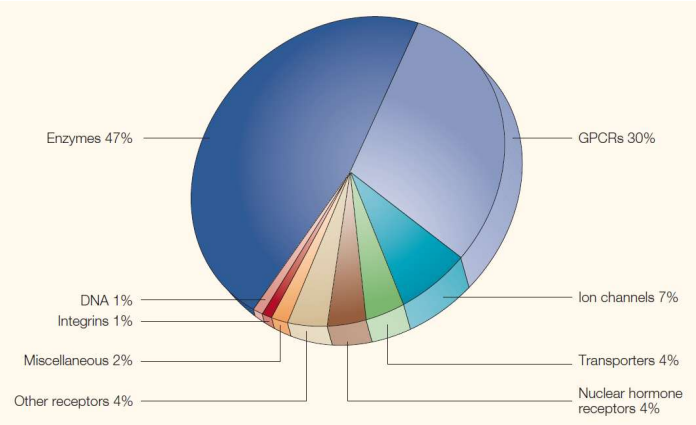
Rational Drug Design lecture 9

Łukasz Berlicki

Drug Molecular targets

- G-protein coupled receptors (GPCR) are one of the main classes of molecular targets.

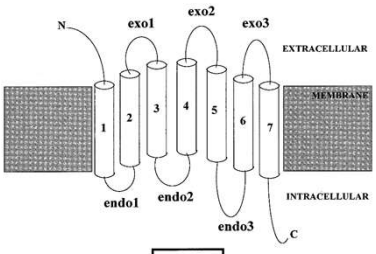


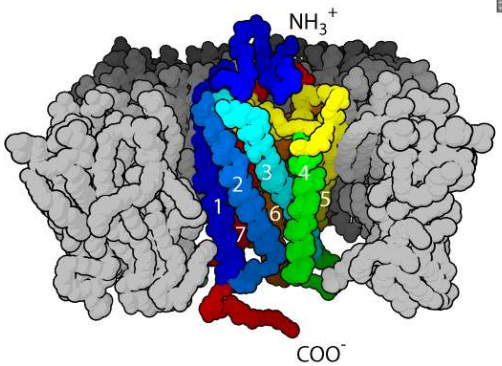
Drugs

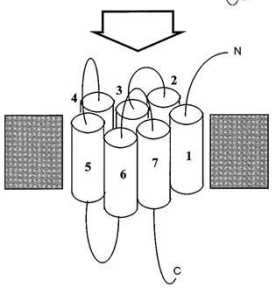
- Approx. 30 GPCR is the drug targets,
- About 240 GPCRs with specific ligands and 160 GPCRs without specific ligands (orphan receptors) are known.
- Approx. 25% of the top 100 block busters are GPCR ligands.
- Drugs that are GPCR ligands are used in a very wide spectrum of diseases, among others: allergies, mental illness, hypertension, cancer, asthma, pain.

Structure of GPCR

- The structure of all GPCRs is similar: 7 transmembrane helices, C-terminus inside, N-terminus outside.



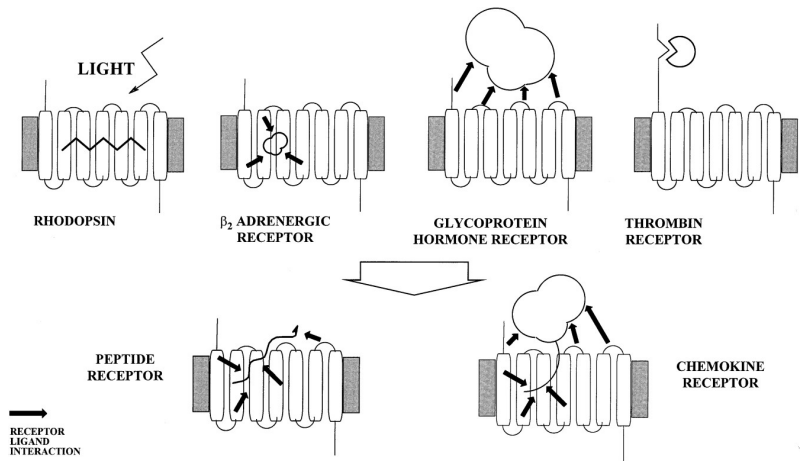




Endogenous ligands

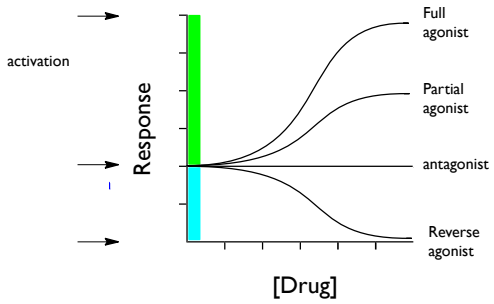
- **Biogenic amines:** adrenaline, dopamine, histamine, acetylcholine, noradrenaline, serotonin, melatonin
- **Peptides and proteins**: angiotensin, bradykinin, bombesin, calcitonin, chemokines, growth hormone, enkephalins and endomorphins, neuropeptide Y, somatostatin, vasopressin and others.
- **Lipid compounds**: anandamide, leukotrienes, prostaglandins, thromboxanes,
- **Nucleotides and purines**: adenosine, cAMP, ATP, UTP, ADP, UDP
- **Amino acids and ions**: glutamate, GABA, Ca^{2+}.

Ligand binding mode

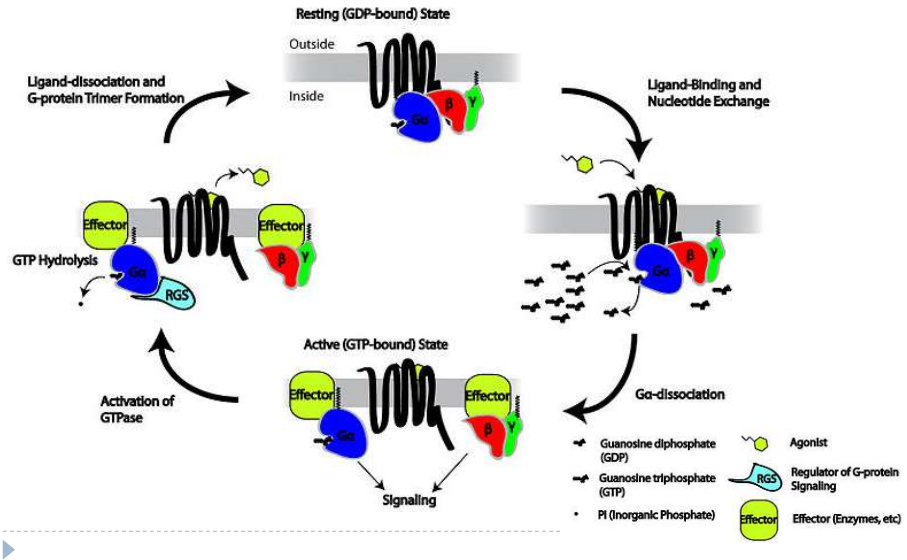


The action of ligands

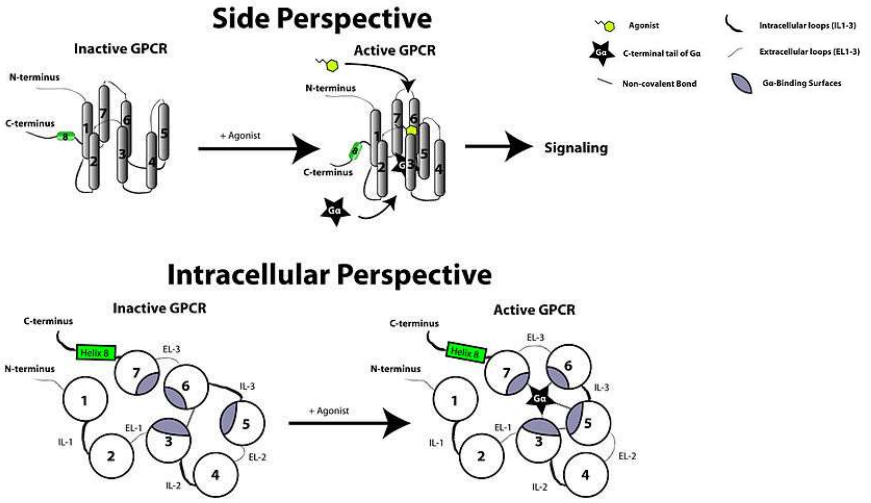
- **An agonist** - a ligand that binds to the receptor causing a pharmacological response (full or partial),
- **Inverse agonist** - a ligand that responds to an endogenous ligand,
- **Antagonist** - a ligand that competes with the endogenous ligand and reduces the pharmacological response.



The action of GPCR



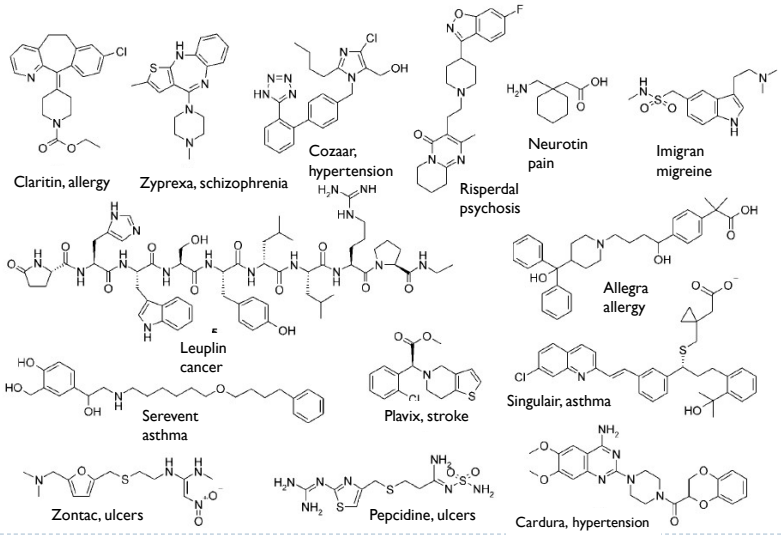
The action of GPCR



GPCR

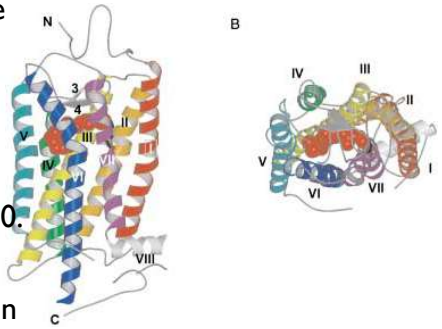
- GPCRs are involved in many important physiological processes:
  - vision,
  - feeling of taste and smell,
  - regulation of behavior and mood,
  - regulation of immune system activity and reaction to inflammation,
  - transmission of signals in the autonomic nervous system,
  - regulation of homeostasis (eg water balance),
  - growth and metastasis of some types of cancer.

Drugs – ligands of GPCR



Structure of GPCR

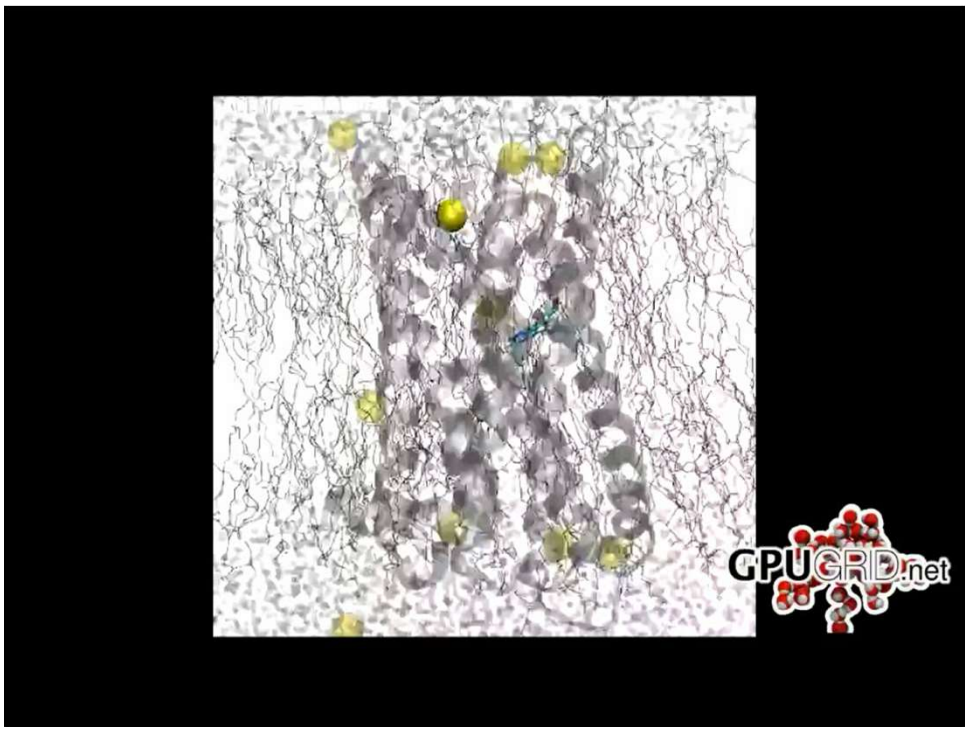
- Structural studies of GPCR are difficult because it is a transmembrane protein.
- The first GPCR - rhodopsin structure was published in 2000.
- The Nobel Prize in chemistry in 2012 was awarded for research on the GPCR: Robert Lefkowitz and Brian Kolibka.



K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, R. E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, 289, 739

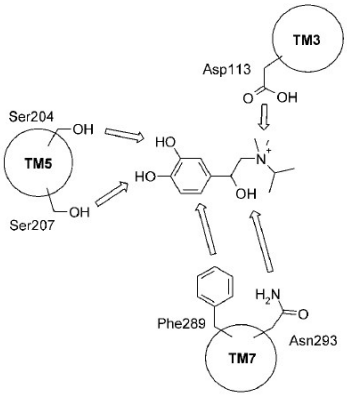
Ligand-based drug design

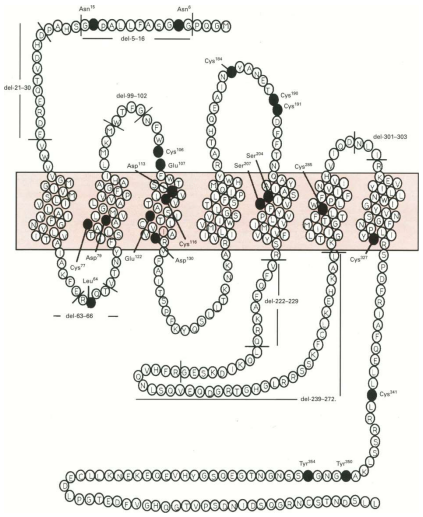
- Due to the very limited knowledge of the GPCR structure, drug design is based mainly on ligand structures (ligand based drug design).
- A good starting point are endogenous ligands.
- The study of the structure-activity dependency relationship is crucial (structure-activity relationships, SAR).
- The model of an endogenous ligand or its analog may be the basis for searching for other structures by applying pharmacophores.



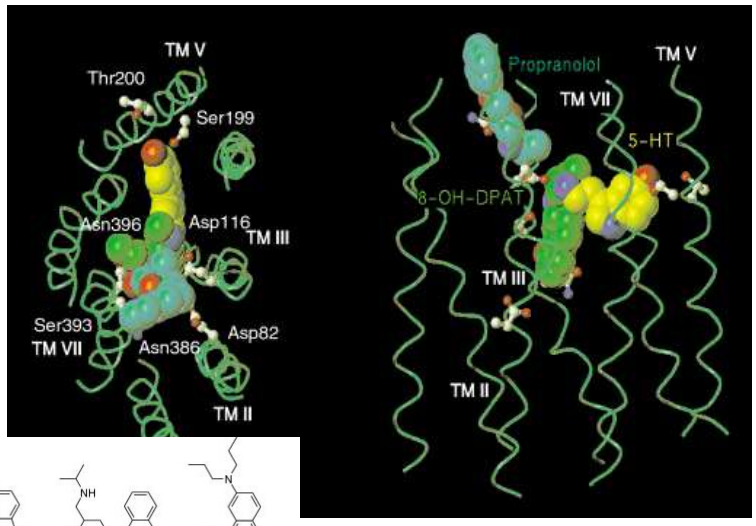
Ligand binding mode

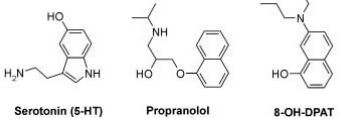
- The ligand binding mode can be tested by site-directed mutagenesis.





Ligand binding mode

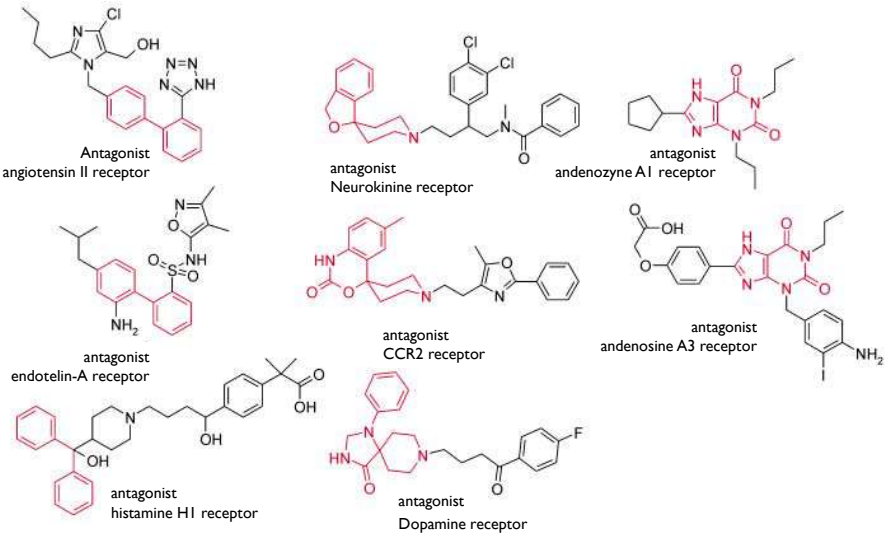




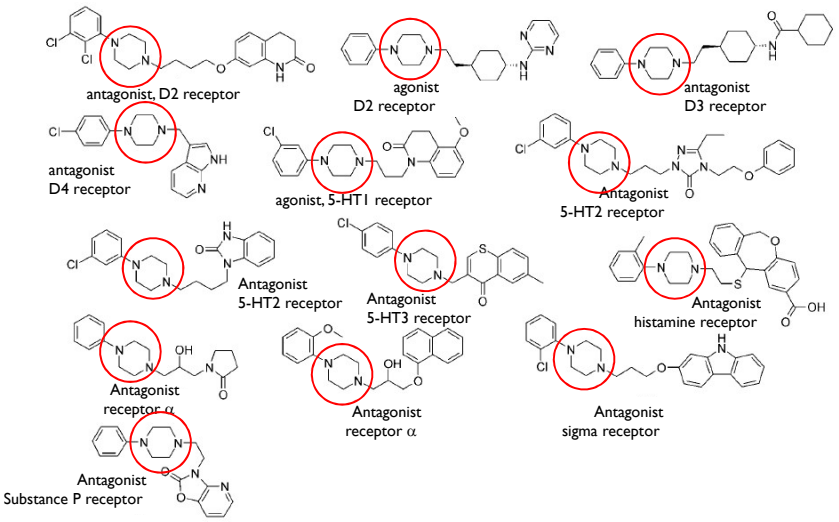
Privileged structures

- Privileged structures are structural fragments that can be the basis for designing and obtaining ligands for various receptors.
- Various modifications of such structures can be an important method for the search for new receptor ligands (agonists and antagonists).
- The existence of privileged structures results from the structural similarity of some elements of the GPCR.

Privileged structures

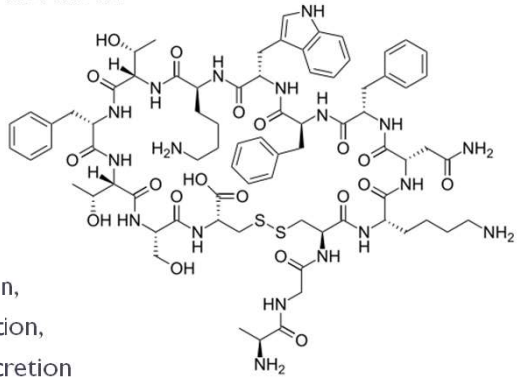


Privileged structures



Somatostatin

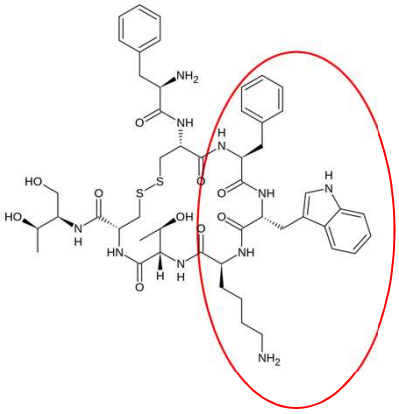
- Somatostatin - a peptide hormone
- Secretion:
  - digestive system
  - brain
- Action:
  - Inhibition of secretion growth hormone,
  - Inhibition of TSH secretion,
  - Inhibition of insulin secretion,
  - Inhibition of hormone secretion gastrointestinal
  - It reduces the rate of emptying the digestive system.



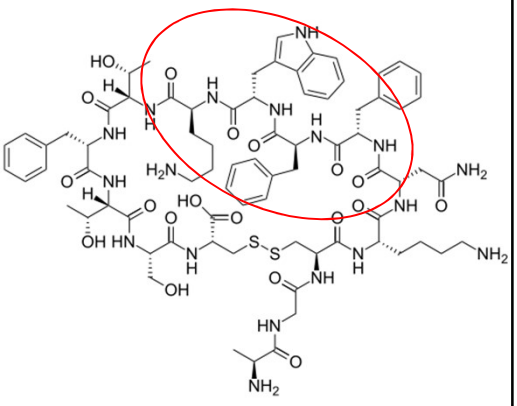
Drugs

- Somatostatin receptor agonists can be medicines for:
  - Acromegaly (excessive secretion of growth hormone due to pituitary adenoma)
  - Carcinoid syndrome (hormonally active cancer)

Drugs

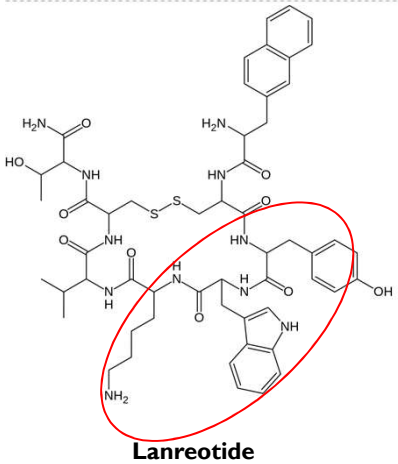


Octreotide
t ½ = ok. 90 min

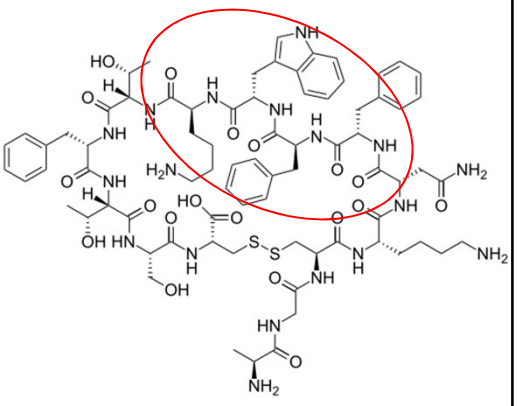


Somatostatin
t ½ = ok. 2-3 min

Drugs

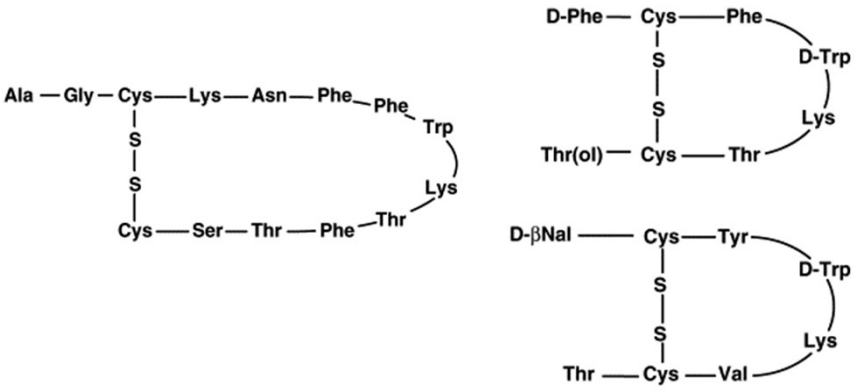


Lanreotide
t ½ = ok. 90 min



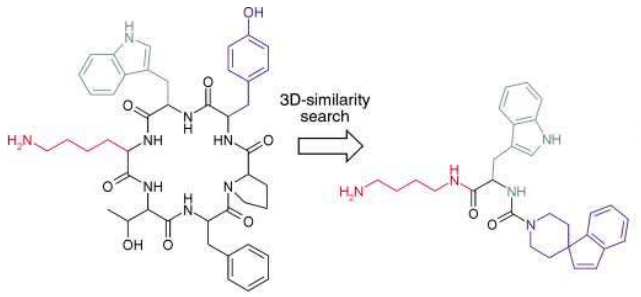
Somatostatin
t ½ = ok. 2-3 min

Somatostatin and analogs



Somatostatin

- The cyclic hexapeptide with the Tyr-D-Trp-Lys motif was used to search the base of compounds to find a compound of similar structure

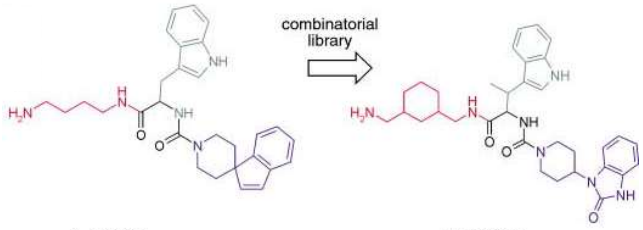


L-363,377

L-264,930
K_i = 100 nM

Somatostatin

- The use of combinatorial chemistry to find selective antagonists of somatostatin receptors



L-264,930

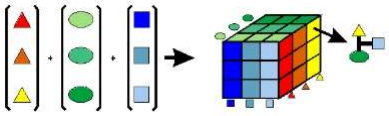
L-779,976

K_i = 0.05 nM

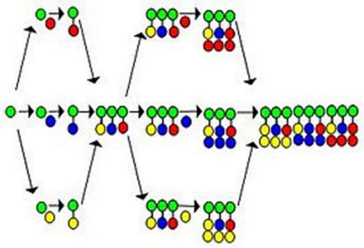
20 diamine substituents
20 Trp derivatives
79 aromatic derivatives
=
130 000 compounds

Combinatorial chemistry

- By testing mixtures of many compounds, a large number of analogs can be quickly screened.

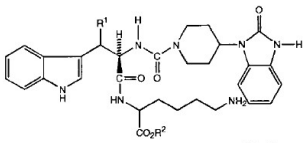


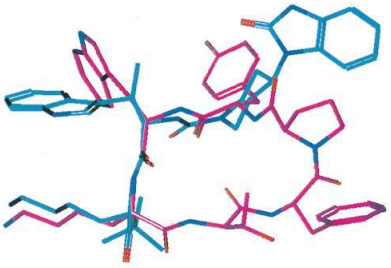
- Testing smaller and smaller groups of compounds allows you to find the most active one.



Somatostatin

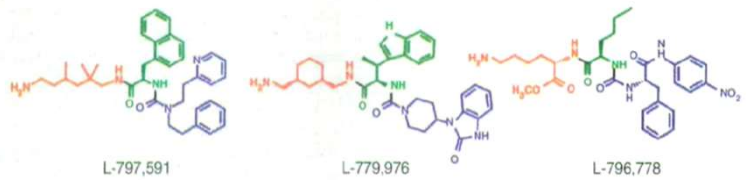
- Optimization of the ligand structure



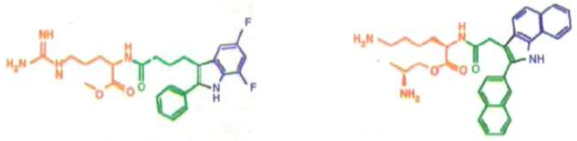


Compound	R1	R2	Ki(nM) h1	h2	h3	h4	h5
4	''''''CH_3	CH_3	5,505	0.46	1,208	4,167	4,658
5	H	$C(CH_3)_3$	1,701	0.1	226.4	397	1,184
6	◄CH_3	$C(CH_3)_3$	4,766	2.1	515.2	379	1,193
7 (L-054,522)	---CH_3	$C(CH_3)_3$	2,392	0.01	31	81	163

Somatostatin



L-797,591 L-779,976 L-796,778

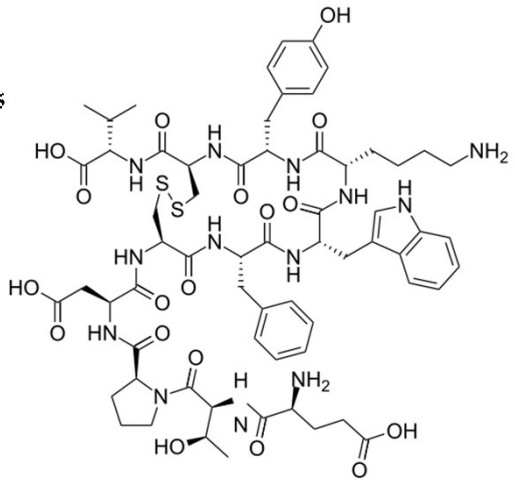


L-803,087 L-817,818

Compound	hsstr1	hsstr2	hsstr3	hsstr4	hsstr5
ss-14	0.4	0.04	0.7	1.7	2.3
L-363,377	5664	0.5	3072	>10,000	2009
L-797,591	1.4	1875	2240	170	3600
L-779,976	2760	0.05	729	310	4260
L-796,778	1255	>10,000	24	8650	1200
L-803,087	199	4720	1280	0.7	3880
L-817,818	3.3	52	64	82	0.4

Urotensin II

- Urotensin II regulates the work of the cardiovascular system.
- The urotensin II receptor is a potential target for drugs against cardiovascular disease.



Urotensin II

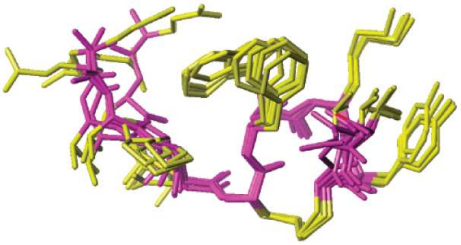
- The alanine scan and the study of short peptides allow to find the motif responsible for activity.
- The key motive is Trp-Lys-Tyr.

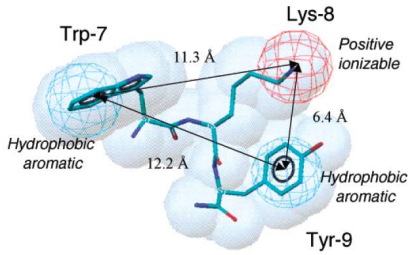
no.	peptide	sequence	EC_{50} (nM)	E_{max}
1	U-II (1–11)	ETPDCFWKYCV	2.5 ± 0.2	100
2	U-II (2–11)	TPDCFWKYCV	2.4 ± 0.1	103 ± 3.8
3	U-II (3–11)	PDCFWKYCV	3.6 ± 0.6	100 ± 5.5
4	U-II (4–11)	DCFWKYCV	3.0 ± 0.9	102 ± 4.5
5	U-II (5–11)	CFWKYCV	1.8 ± 0.3	106 ± 4.0
6	U-II (1–10)	ETPDCFWKYC	1.8 ± 0.1	99 ± 3.5
7	U-II (5–10)	CFWKYC	2.3 ± 1.1	95 ± 6.8

no.	sequence	EC_{50} (nM)	E_{max}
8	**A**TPDCFWKYCV	1.8 ± 0.3	101 ± 1.7
9	E**A**PDCFWKYCV	3.3 ± 0.8	101 ± 0.7
10	ET**A**DCFWKYCV	1.9 ± 0.8	102 ± 3
11	ETP**A**CFWKYCV	2.7 ± 0.5	101 ± 3
12	ETPD**A**FWKYCV (linear)	233 ± 16	96 ± 4
13	ETPDC**A**WKYCV	5.7 ± 0.3	98 ± 1
14	ETPDCF**A**KYCV	1,303 ± 98	45 ± 2
15	ETPDCFW**A**YCV	14,800 ± 800	ND
16	ETPDCFWK**A**CV	193 ± 9	75 ± 3
17	ETPDCFWKYC**A**	3.2 ± 0.3	99 ± 2

Urotensin II

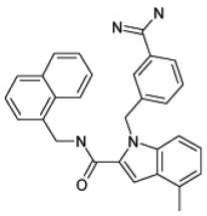
- Peptide conformation allows the determination of formacophore.

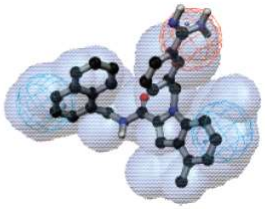




Urotensin II

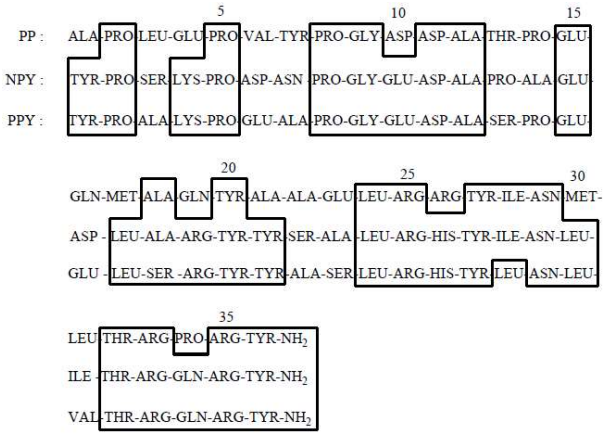
- Searching the database of compounds and establishing the 3D similarity gives a chance to find an active antagonist





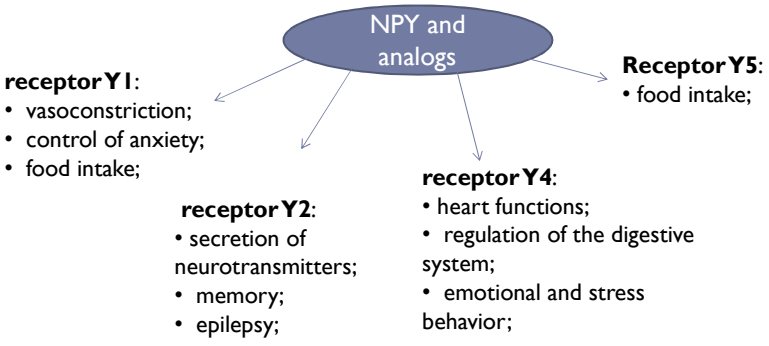
Neuropeptide Y and analogs

- Neuropeptide Y (NPY), pancreatic polypeptide (pp) and polypeptide Y (PPY) are peptide hormones secreted from the brain and autonomic nervous system that act on Y receptors.



Neuropeptide Y and analogs

- Interaction with various Y receptors (Y1-Y5) controls various functions.

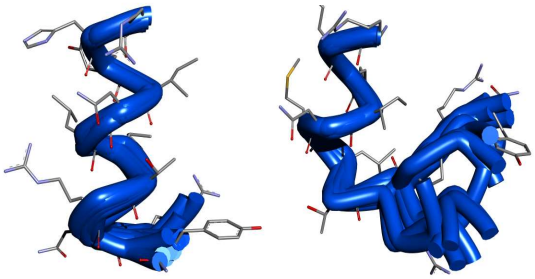


Y receptor ligands as drugs

- The agonists of the Y1 and Y5 receptors cause **increased** food intake, and the Y2 and Y4 receptor agonists **reduce** food intake.
- Y1 and Y5 antagonists or Y2 and Y4 agonists can be effective obesity lacunae.
- The development of selective ligands is necessary.

Neuropeptide Y and analogs

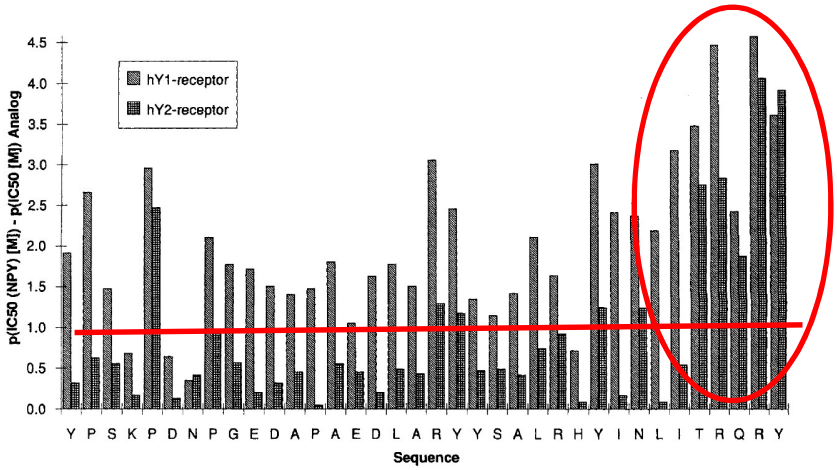
- The structure of NPY and PP is similar - unordered N-terminus and helical C-terminus



pNPY bPP

Peptide	Y_1 IC_{50} [nM]	Y_2 IC_{50} [nM]	Y_4 IC_{50} [nM]	Y_5 IC_{50} [nM]
pNPY	0.2	0.04	5.5	0.6
hPP	>100	>1000	0.04	27[a]

Neuropeptide Y – alanine scan



Neuropeptide Y

- The C-terminal NPY fragment retains significant biological activity

compound	rat brain (Y_2) IC_{50} (μM)
1, NPY	0.00045 ± 0.00005
2, NPY(13-36)	0.015 ± 0.002
3, PheMetArgPhe-NH_2	40 ± 1.4
4, ThrArgGlnArgTyr-NH_2	>100
5, IleTyrArgLeuArgTyr-NH_2	2.95 ± 1.7
6, LeuIleTyrArgLeuArgTyr-NH_2	0.4 ± 0.01
7, AsnLeuIleTyrArgLeuArgTyr-NH_2	0.31 ± 0.01
8, IleAsnLeuIleTyrArgLeuArgTyr-NH_2	0.037 ± 0.006
9, TyrIleAsnLeuIleTyrArgLeuArgTyr-NH_2	0.008 ± 0.003
10, HisTyrIleAsnLeuIleTyrArgLeuArgTyr-NH_2	0.012 ± 0.002
11, ArgHisTyrIleAsnLeuIleTyrArgLeuArgTyr-NH_2	0.012 ± 0.007

Neuropeptide Y

- Simple modifications (dimerization, substitution) of short peptides can increase selectivity.

peptide	Y1	Y2
NPY	0.52 ± 0.02	0.23 ± 0.02
1[c]	5.0 ± 0.46	11.3 ± 3.8
2	25.7 ± 5.9	1420 ± 191
3	34.8 ± 5.8	1650 ± 114
4	4.8 ± 1.1	1120 ± 15
5	2.3 ± 0.5	822 ± 78
6[c]	0.07 ± 0.01	55 ± 21
7	0.27 ± 0.01	1036 ± 116
8	0.46 ± 0.12	624 ± 69

Ile–Asn-Pro-Ile-Tyr-Arg-Leu-Arg-Tyr-NH_2

Ile–Asn-Pro-Ile-Tyr-Arg-Leu-Arg-Tyr-OMe

Ile–Asn-Pro-Ile-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2

Ile–Asn-Pro-Cys-Tyr-Arg-Leu-Arg-Tyr-OMe

Ile–Asn-Pro-Cys-Tyr-Arg-Leu-Arg-Tyr-OMe

Ile–Asn-Pro-Cys-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2

Ile–Asn-Pro-Cys-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2

Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-NH_2

Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-NH_2

Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-OMe

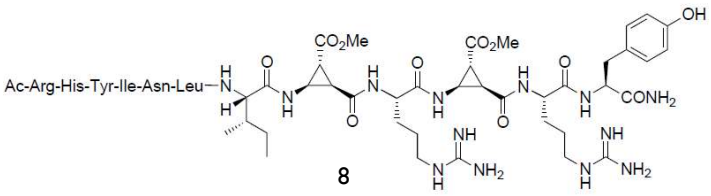
Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-OMe

Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2

Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2

Neuropeptide Y

- The stiffening of the peptide structure increases its selectivity.



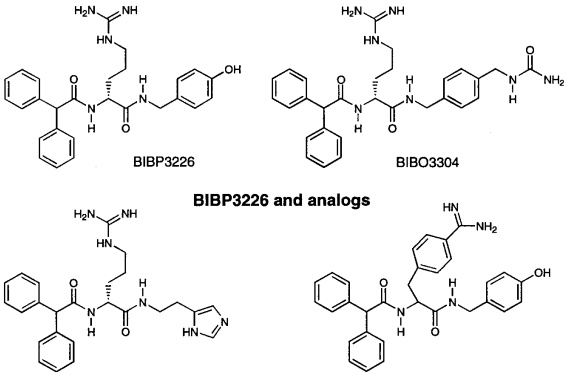
8

	Sequences[b]	Y_1	Y_2	Y_5
	RHYINLITRQRY-NH_2	>1000	21[d]	>1000
4	RHYINLITR▲RY-NH_2	37(±20)	>1000	724
5	RHYINLITR▼RY-NH_2	>1000	>1000	>1000
6	RHYINLI▲RQRY-NH_2	>1000	>1000	>1000
7	RHYINLI▼RQRY-NH_2	>1000	>1000	>1000
8	RHYINLI▲R▲RY-NH_2	50(±10)	>1000	617

Neuropeptide Y

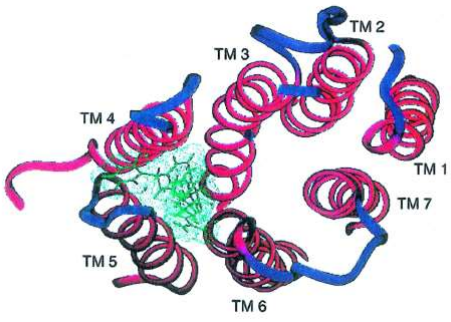
- Non-peptide ligands based on the C-terminal NPY fragment

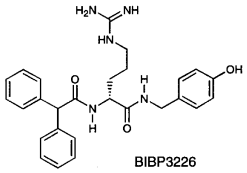
Asn-Leu-Ile-Thr-Arg-**Asn-Arg-Tyr-NH_2**



Neuropeptide Y

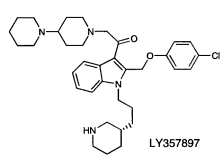
- A mode for binding a non-peptide BIBP3226 antagonist to a receptor

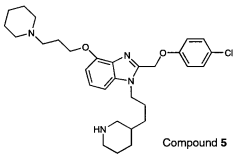


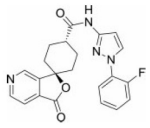


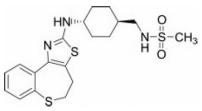
Neuropeptide Y

- Other non-peptide Y receptor ligands

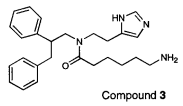


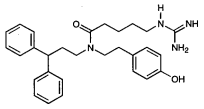


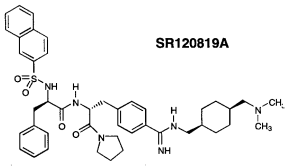


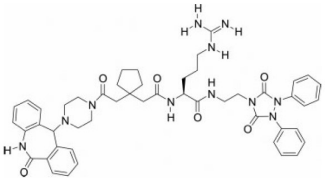


ω-Amino- and ω-Guanidino-alkanamides



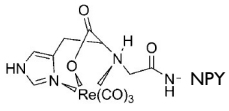


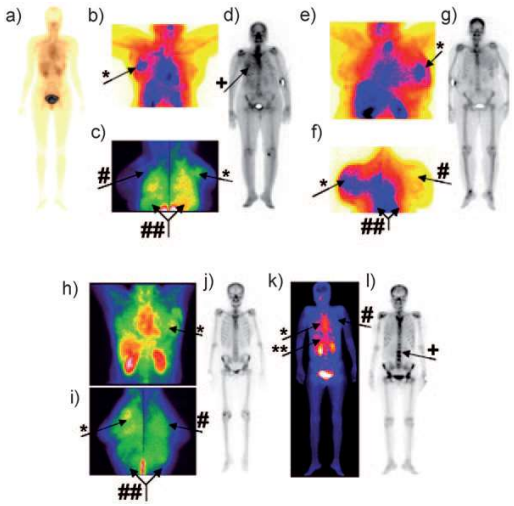




Neuropeptide Y

- Y1 receptors are overexpressing in breast cancer.
- The combination of NPY with an isotopic marker allows tumor imaging.





Summary

- G-protein-coupled receptor ligands are an important class of drugs because these receptors control many important processes.
- The design of GPCR agonists and antagonists is based mainly on the structure of ligands.