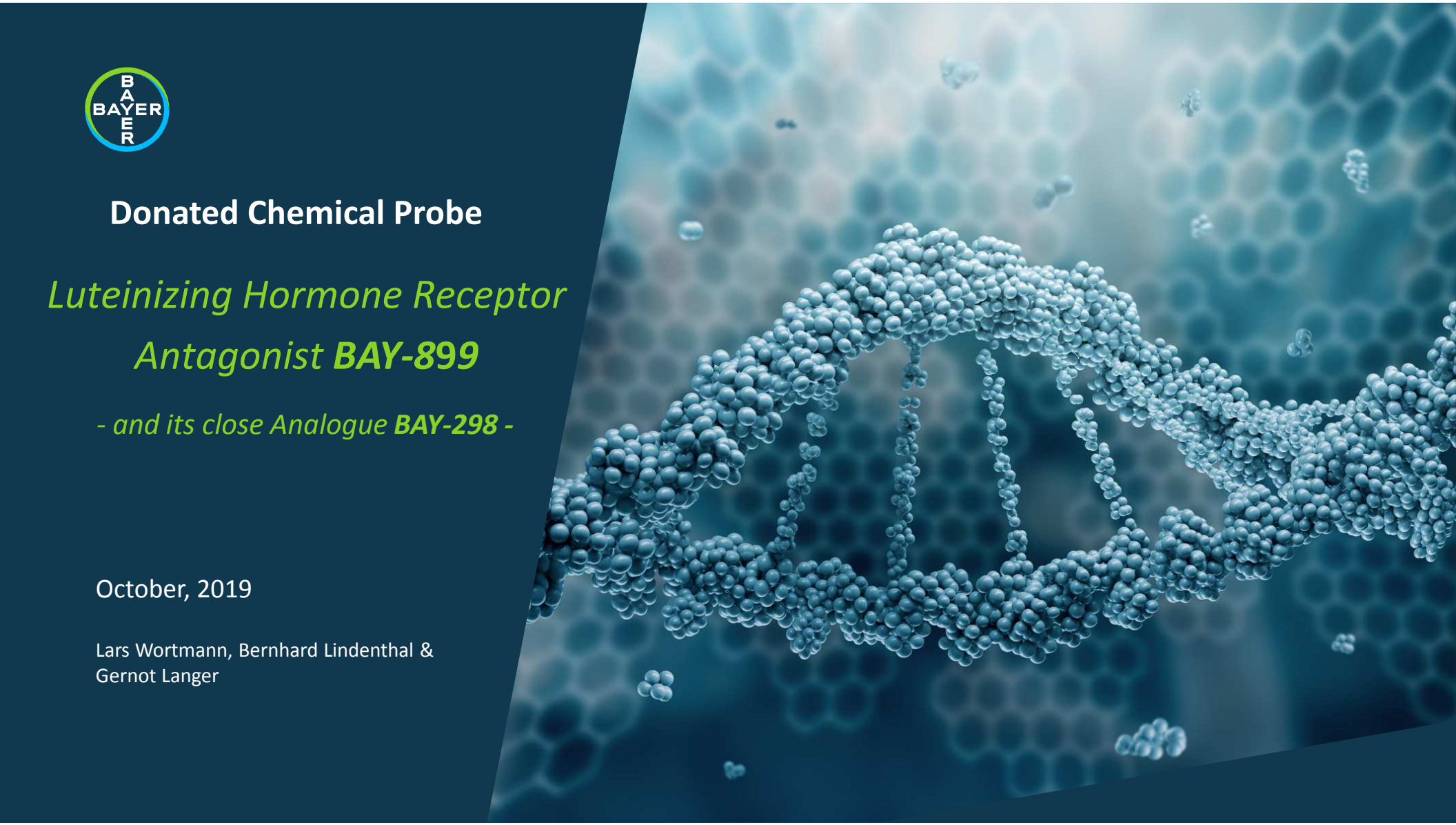


Donated Chemical Probe

*Luteinizing Hormone Receptor Antagonist **BAY-899***

*- and its close Analogue **BAY-298** -*

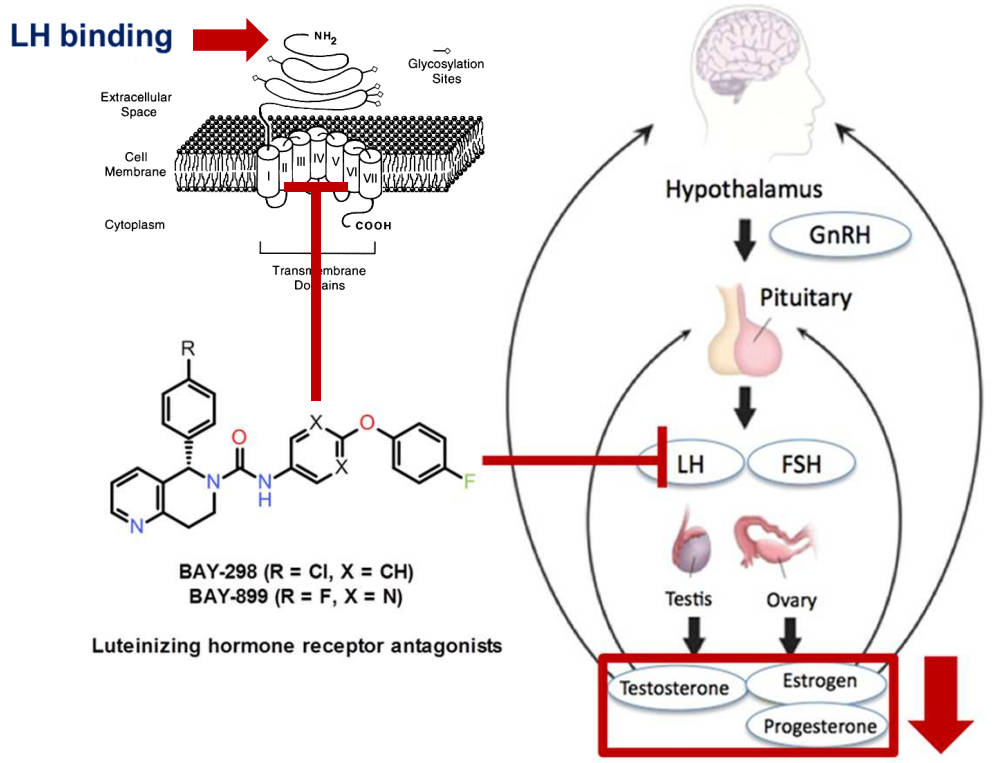
October, 2019

Lars Wortmann, Bernhard Lindenthal &
Gernot Langer

Luteinizing Hormone Receptor (hLH-R)

Target rationale

- **LH-R** belongs to the family of **glycoprotein hormone GPCRs** (together with FSH- and TSH-R). All three signal into the **cAMP** second messenger pathway
- **LH-R** is **activated by** high affinity **agonist LH** binding to a large, glycosylated extracellular domain
- **LH** is a non-covalently linked, **heterodimeric glycoprotein** consisting of an **α- and a β-subunit;** (α-subunit common to all glycoprotein hormones)
- **LH** and **FSH** are key components of the hypothalamic–pituitary–gonadal **(HPG) axis** and **essential for** the **regulation of sex hormone levels** in males and females
- **LH-R** is a **valid target for** intervention of **hormone-dependent diseases** and for contraception



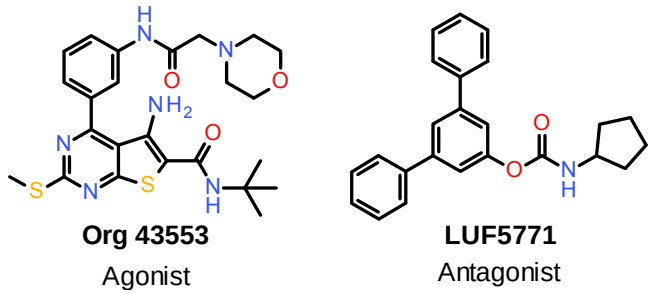
Modified from http://www.dsdgenetics.org and used with permission of Prof. Peter Koopman.

LH-R Antagonists are of great interest when studying hormone-dependent diseases, contraception or other reproductive biological processes

Luteinizing Hormone Receptor Antagonists

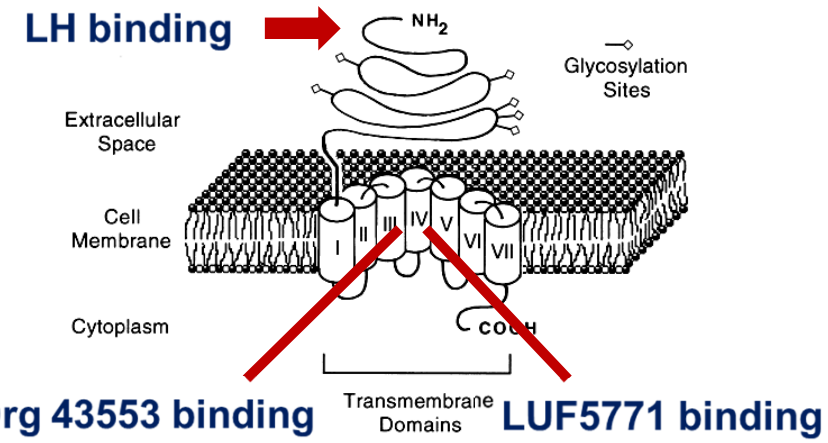
SMOL modulators of hLH-R activity known from literature

- Two SMOL ligands of the hLH-R described in the literature: **Org 43553 (Agonist)** & **LUF5771 (Antagonist)**
- **Org 43553 activates** the hLH-R by reversibly binding to an **allosteric binding site, i.e. without interfering with the binding of the natural ligand LH**
- **LUF5771 antagonizes** hLH-R signaling by reversibly binding to a **second allosteric site (i.e. distinct from the Org 43553 binding pocket *)** - without interfering with the binding of the natural ligand LH again.
- **LUF5771 is not suitable for in vivo application**



Org 43553
Agonist

LUF5771
Antagonist

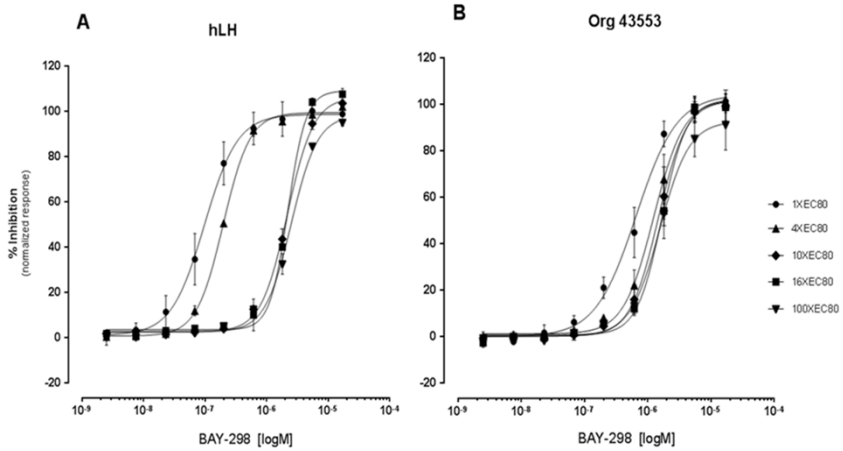


** Heitmann, L.H. et al. MCE, 351, (2012), 326 - 336*

No hLH-R antagonist suitable for *in vivo* application is described in the literature

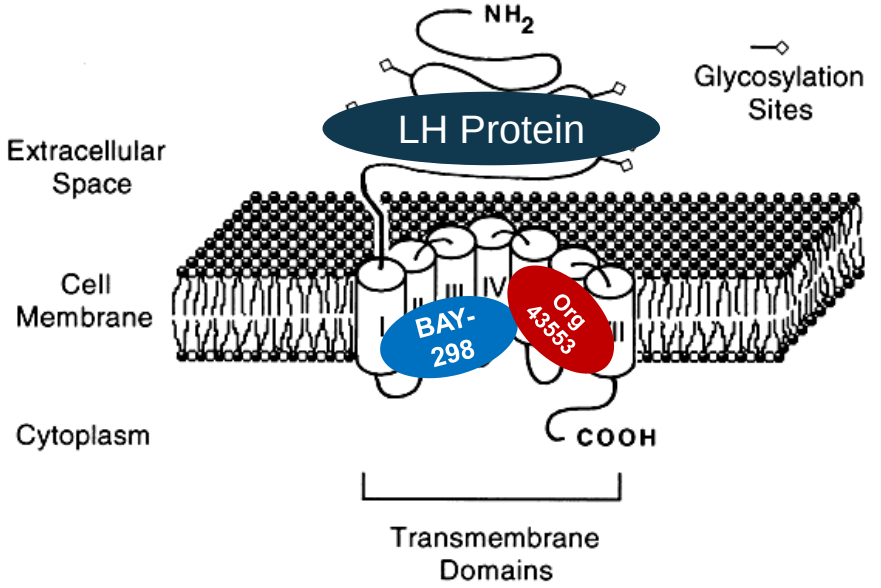
Luteinizing Hormone Receptor Antagonists

*Antagonist dose response studies: **BAY-298** mode of action*



- Limited rightward shift observed in the presence of either LH or the SMOL agonist Org43553 -> **BAY-298** behaves as a **non-competitive hLH-R antagonist** (comparable results expected for actual probe BAY-899 ~ highly structurally related)
- Working hypothesis: Like Org 43553 - **BAY-298 & BAY-899 bind to remnants of the rhodopsin binding site of hLH-R**

Illustrative: Binding modes for LH protein, **Org43553** and **BAY-298**



BAY-298 (and, in all likelihood, **BAY-899**) act as a non-competitive, i.e. allosteric hLH-R antagonist(s)

Luteinizing Hormone Receptor Antagonists

*In vitro Profile of **BAY-298** and SGC-Donated Chemical Probe **BAY-899***

Pharmacological *in vitro* Properties	
h LH Antagonism, IC_{50}	96 nM
r LH Antagonism, IC_{50}	23 nM
cyn LH Antagonism, IC_{50}	78 nM
h TSH Antagonism, IC_{50}	2.3 µM
h FSH Antagonism, IC_{50}	> 16 µM
h LH Agonism, EC_{50}	> 16 µM
h Fraction unbound [%]	0.2

Safety Properties	
hERG [µM]	3.0

33 (BAY-298)

Luteinizing hormone receptor antagonist

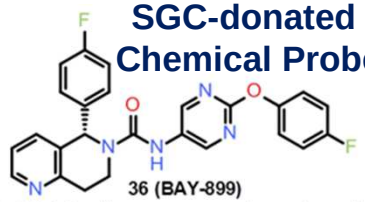
Physicochemical Properties	
MW corr [g*mol]	474
TPSA [Å²]	54
LogD @pH 7.5	4.7
Sw pH 6.5 [mg/L]	3.5
Chemical stability, pH	stable

In vitro DMPK Properties						
Caco2 permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	15		20		1.3	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver microsomes (h /r)		0.2 / 0.2		85 / 94	
	hepatocytes (r)		1.1		74	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 20	0.7	4.4	> 20	> 20	> 20

SGC-donated Chemical Probe

Pharmacological *in vitro* Properties	
h LH Antagonism, IC_{50}	185 nM
r LH Antagonism, IC_{50}	46 nM
cyn LH Antagonism, IC_{50}	n.d.
h TSH Antagonism, IC_{50}	24 µM
h FSH Antagonism, IC_{50}	> 16 µM
h LH Agonism, EC_{50}	n.d.
h Fraction unbound [%]	1.7

Safety Properties	
hERG [µM]	10.6



36 (BAY-899)

Luteinizing hormone receptor antagonist

Physicochemical Properties	
MW corr [g*mol]	459
TPSA [Å²]	80
LogD @pH 7.5	2.8
Sw pH 6.5 [mg/L]	39
Chemical stability, pH	stable

In vitro DMPK Properties						
Caco2 permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	89		95		1.1	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver microsomes (h /r)		0.1 / 0.2		89 / 95	
	hepatocytes (r)		1.3		68	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 20	3.1	9.9	> 20	> 20	> 20

BAY-899 was selected as SGC-donated Chemical Probe due to its better *in vitro* DMPK, PhysChem and selectivity.

Luteinizing Hormone Receptor Antagonists

*Functional Selectivity Profile of **BAY-899***

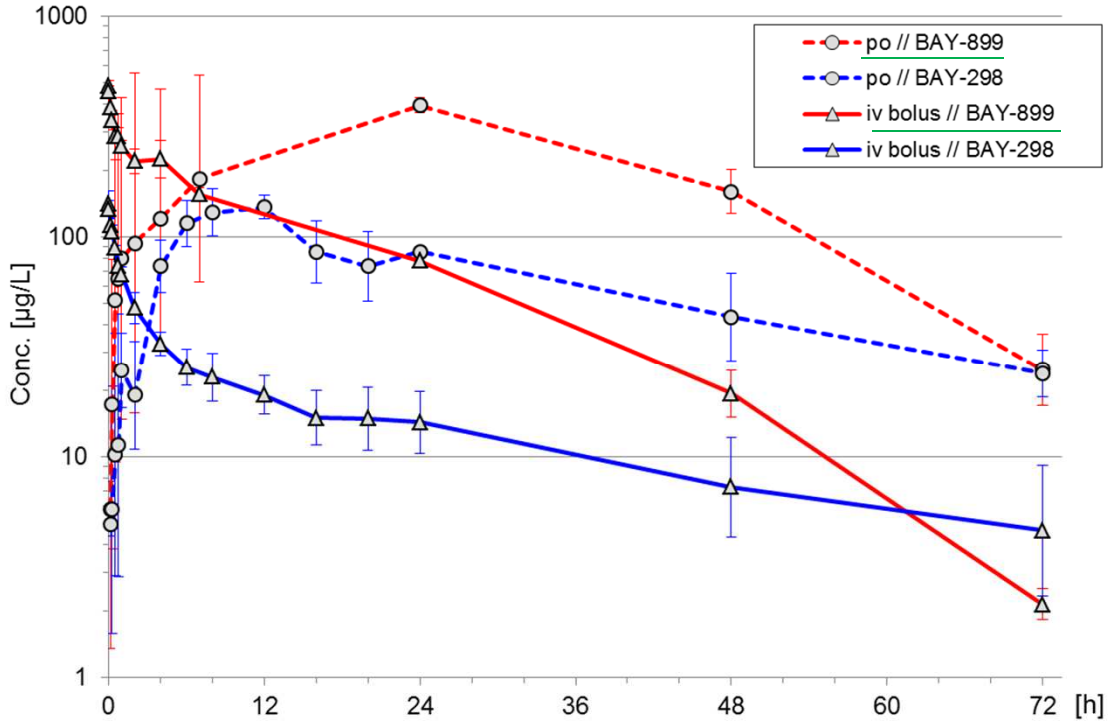
- **Functional selectivity profile of BAY-899 in panel of 25 GPCRs** (“Bayer-Panel” @ Eurofins)
- **Both, the antagonistic (inhibition) and agonistic (activation) properties were determined**
- The percentage of the mean value obtained when testing in duplicates at a single concentration of **10 µM is reported.**
- Conclusion: No inhibition or activation > 70% at 10 µM compound concentration reveals **excellent selectivity within target family**

	Inhibition (% of control)	**Activation** (% of control)
A2B	16	-1
A3	-6	25
alpha 1A	45	2
alpha 2A	-16	-2
beta 1	13	-1
beta 2	26	-3
CB1	4	52
D1	15	-1
D2L	67	-1
H1	4	-4
H2	34	-2
H3	-19	38
MC4	11	-2
Motilin	28	-1
M1	32	1
M4	2	-6
NK1	24	-3
kappa (KOP)	-10	56
mu (MOP)	8	56
EP3	-5	-1
P2Y2	8	-1
5-HT1A	0	1
5-HT2B	18	1
5-HT6	12	-1
sst4	6	23

BAY-899 is highly selective in a commercial panel of 25 GPCRs (tested in agonistic and antagonistic mode)

Luteinizing Hormone Receptor Antagonists **BAY-298** & **BAY-899**

Pharmacokinetics (Low dose rat PK)



BAY-899			
Admin Route		iv bolus	po
Dose Admin	[mg/kg]	0,50	2,0
AUC_{norm}	[kg·h/L]	9,4	7,8
$C_{max,norm}$	[kg/L]	0,97	0,24
CL_{matrix}	[L/h/kg]	0,11	
t_{max}	[h]	0,0	24
V_{ss}	[L/kg]	1,8	
$t_{1/2}$	[h]	11	12
F	[%]		83

BAY-298			
Admin Route		iv bolus	po
Dose Admin	[mg/kg]	0,50	2,0
AUC_{norm}	[kg·h/L]	2,5	2,7
$C_{max,norm}$	[kg/L]	0,28	0,066
CL_{plasma}	[L/h/kg]	0,41	
t_{max}	[h]		8,0
V_{ss}	[L/kg]	16	
$t_{1/2}$	[h]	31	33
F	[%]		108

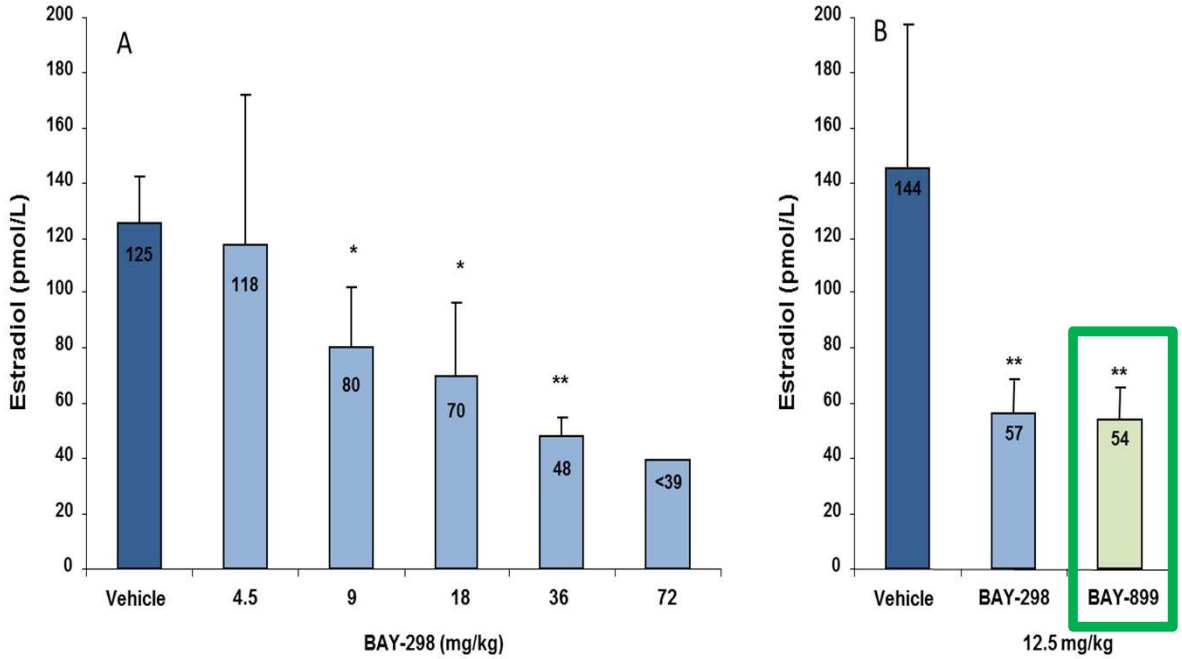
Chemical probe **BAY-899** shows high exposure, long half-life, high oral bioavailability and high volume of distribution

Luteinizing Hormone Receptor Antagonists

*In vivo Efficacy Studies with **BAY-298** & **BAY-899***

- **BAY-298** treatment leads to a dose-dependent lowering of estradiol levels in female rats following treatment for 8 days (q.d., po, vehicle: Myrj53 (Polyoxyethylene (50) stearate) in 0.9% w/v NaCl solution (85 mg/100 mL))
- **BAY-899 is equi-efficacious**
- **BAY-298 also showed efficacy in several other *in vivo* studies**

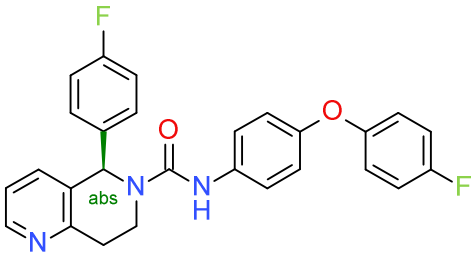
for further information see: Wortmann et al., *J Med Chem.* 2019; 62(22):10321-10341



BAY-899 showed *in vivo* efficacy in a female rat model with regard to the lowering of estradiol levels

Luteinizing Hormone Receptor Antagonists

*Negative Probe **BAY-897***



BAY-897
(R)-enantiomer
IC_{50} (hLH-R) > 16 µM
IC_{50} (hFSH-R) > 16 µM

Solubility (nephelometric): 5 mg/L
Caco-2 permeability: A-B: 12 nm / sec, efflux ratio: 0.4

- **BAY-897** was selected as negative probe molecule
- **BAY-897** showed no activity on the hLH and hFSH receptor

BAY-897 was selected as negative probe molecule and is inactive on the hLH-R and hFSH-R (> 100 fold)

Luteinizing Hormone Receptor Antagonists

*Summary for **BAY-899** & Conclusion*

Probe criteria	BAY-899
Selectivity within target family: goal > 30-fold (based on cellular IC_{50}, Kd)	**Surpasses criteria** No activity on FSH-R, TSH-R. **Clean in commercial GPCR panel of 25 GPCRs** (all < 70% inhibition / activation at 10µM compound concentration)
Selectivity outside target family: describe the off-targets	Lead Profiling Screen for **BAY-899** to be initiated after probe-acceptance. Bayer-internal kinase panel initiated.
On target cell activity for cell-based targets: goal < 1 µM	**Surpasses criteria** 185 nM in cell-based assay
Negative control: *in vitro* potency → ~100-fold less than probe	**Surpasses criteria** **BAY-897** is inactive on hLH-R and hFSH-R
Suitability as *in vivo* chemical probe	**Suitable for *in vivo* experiments**
Publication of BAY-298 & BAY-899 data	Wortmann et al, *J Med Chem.* 2019; 62(22):10321-10341

Luteinizing Hormone Receptor Antagonists

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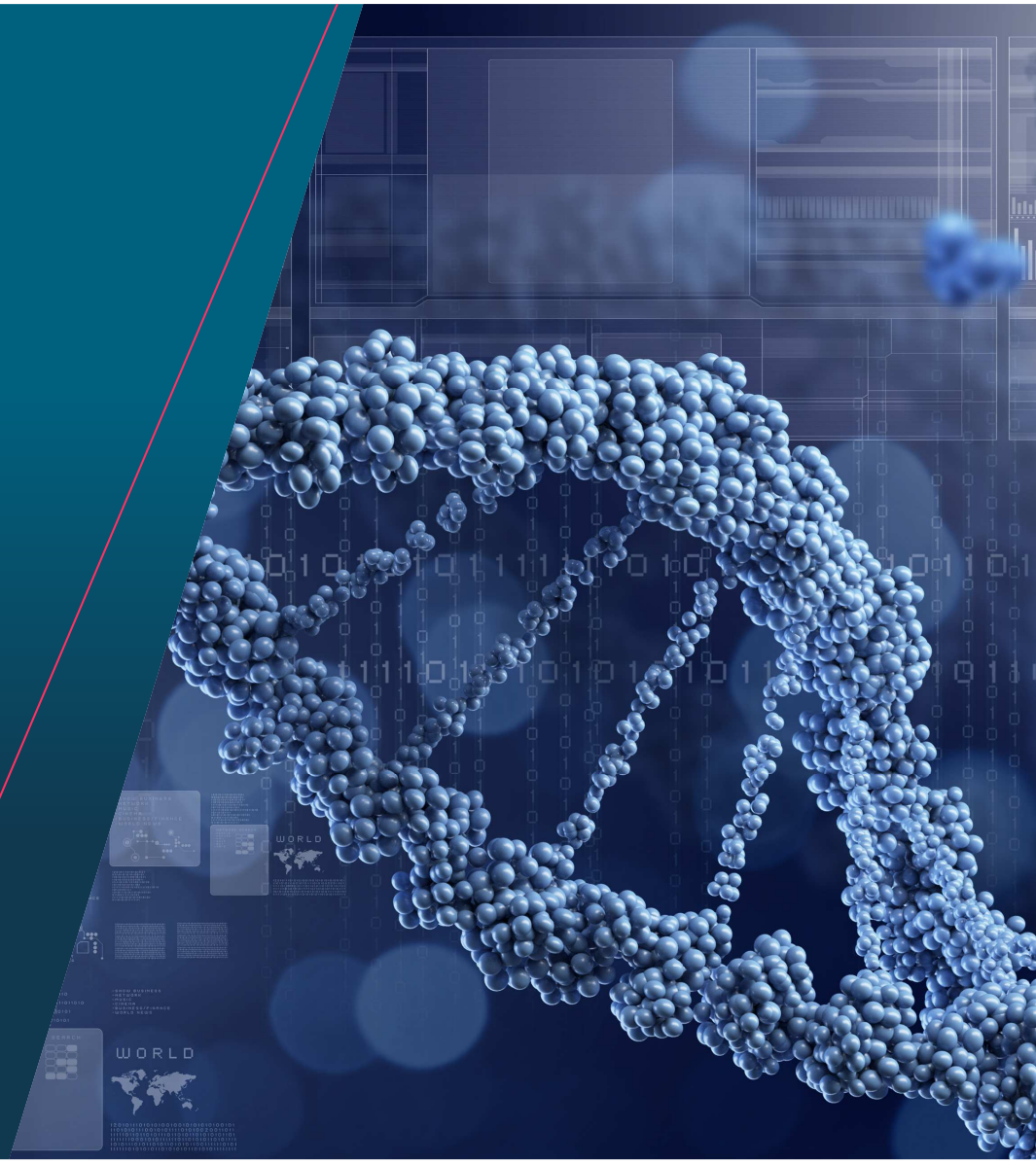
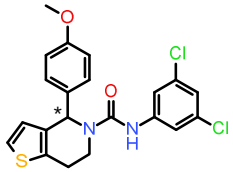
Thank you to the whole team!

Thank You

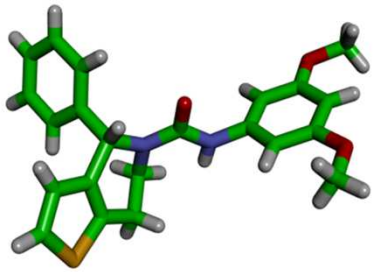


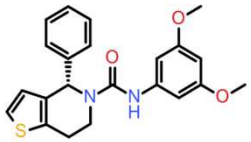
Luteinizing Hormone Receptor Antagonists

Elucidation of the Absolute Configuration of the Eutomer



Compound	Absolute configuration	IC_{50} [nM]				
		hLH	rLH	mLH	hFSH	hTSH
1	racemate	378	212	>30000	>16500	>30000
1a	(*S*)-(+)-enantiomer	403	412	>30000	>16500	>30000
1b	(*R*)-(–)-enantiomer	6790	4890	>30000	>16500	>30000





IC_{50} (hLH) = 193 nM

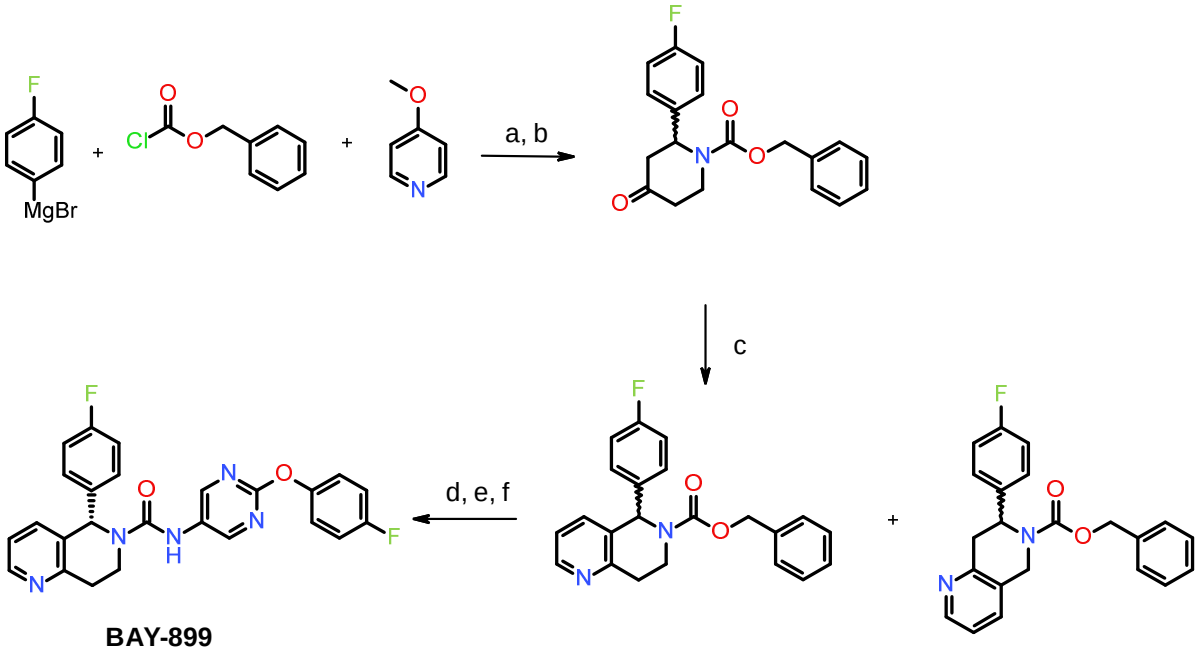
5

- For most of the examples investigated there is a **~ 10 fold difference in the activity of both enantiomers**
- X-ray analysis of compound **5** revealed the **(S)-configuration of the eutomer**

X-ray analysis of compound **5** revealed the (S)-configuration of the eutomer

Luteinizing Hormone Receptor Antagonists

*Synthesis of **BAY-899***



[a]Reagents and conditions: (a) THF; (b) zinc, acetic acid, quantitative; (c) propargylamine, sodium tetrachloroaurate dihydrate, EtOH (as a mixture of regioisomers); (d) TFA, (as a mixture of regioisomers); (e) 4-nitrophenyl chloroformate, 2-(4-fluorophenoxy)pyrimidin-5-amine, THF; (f) HPLC separation of regioisomers and enantiomers.

The synthesis of **BAY-899** is feasible. Separation of regio- and enantiomers requires HPLC.