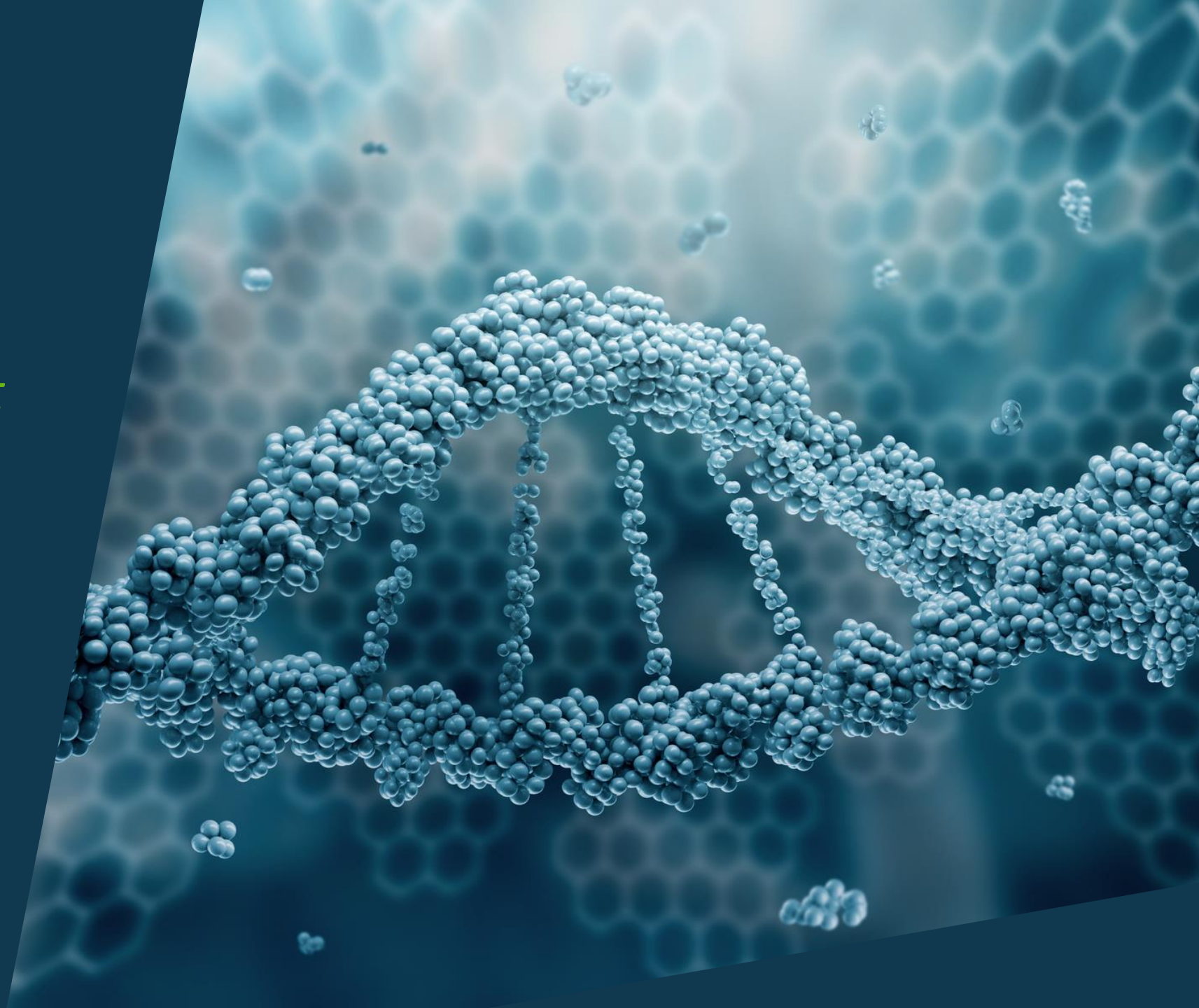
# Donated Chemical Probe

## *GnRH-R Antagonist Probe BAY-784*



June, 2018

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# GnRH-R Ant. Probe BAY-784

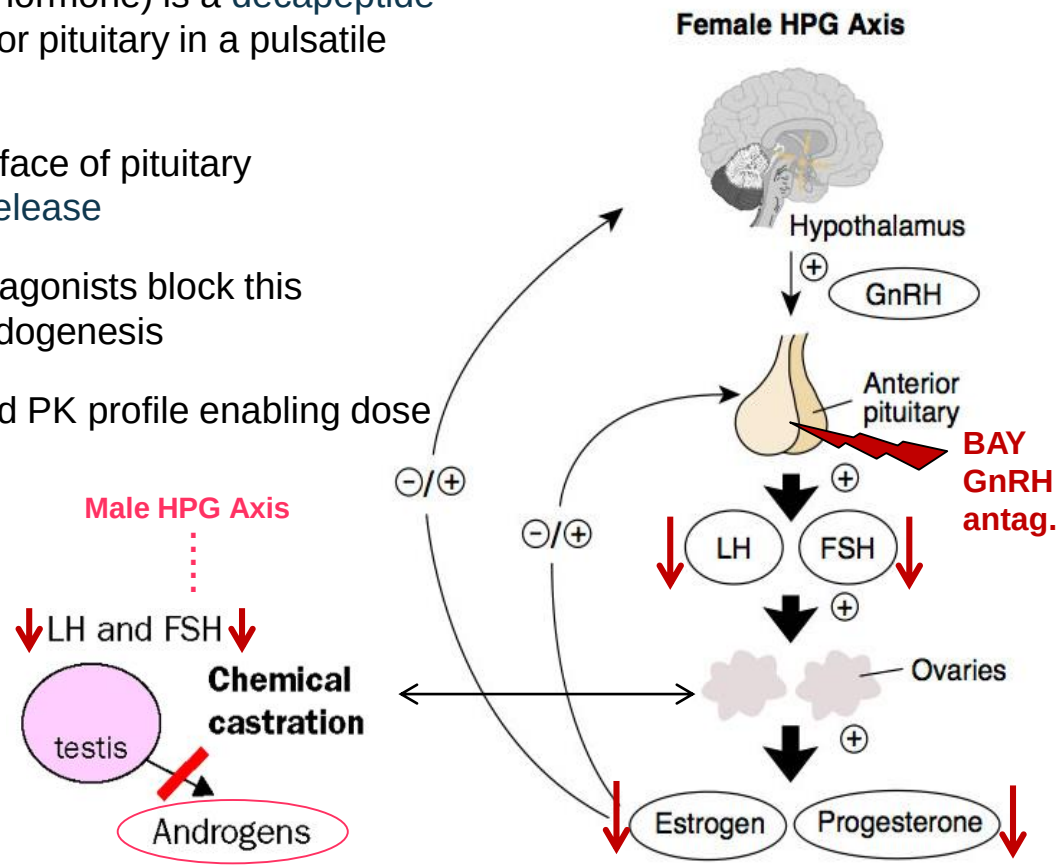
## Scientific Rationale: GnRH as Central Player in HPG-Axis

**// Rationale:** GnRH (gonadotropin releasing hormone) is a decapeptide released from the hypothalamus to the anterior pituitary in a pulsatile manner

// GnRH activates GnRH-R located at the surface of pituitary gonadotrophic cells → triggering LH & FSH release

// GnRH superagonists ( to be injected) & antagonists block this process → leading to impaired ovarian steroidogenesis

**// Orally** available GnRH antagonist with good PK profile enabling dose titration

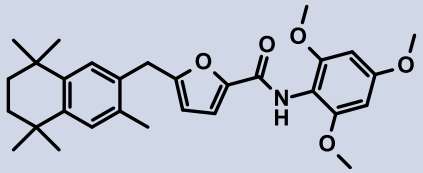
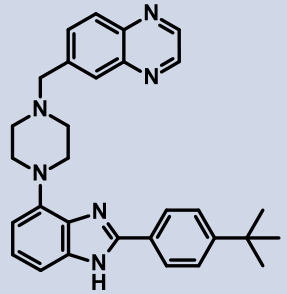
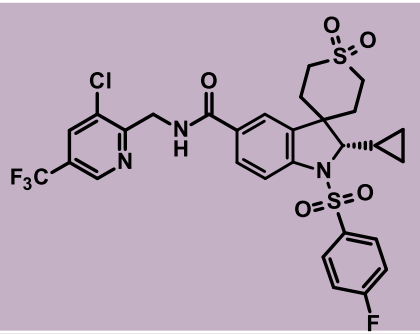



GnRH antag. – similar effect in male HPG axis / impaired testicular androgen production and spermatogenesis

*adapted from Friedman et al., 1990*

# GnRH-R Ant. Probe BAY-784

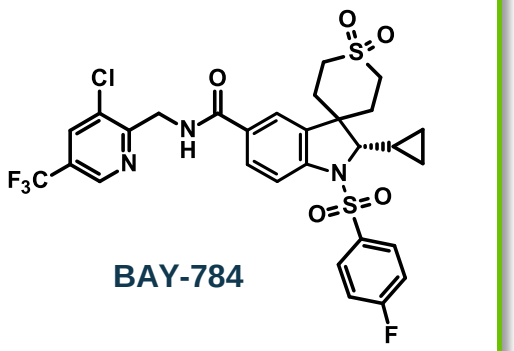
## Two Commercially Available SMOL GnRH Antagonists with Activity at Rodent Receptor

| Company / Identifier | Pfizer: AG 045572 | Wyeth: WAY-207024 | Bayer: BAY-784 |
|---|---|---|---|
| Structure |  |  |  |
| CAS No. | [263847-55-8] | 2HCl: [872002-73-8] | [1631164-24-3] |
| Solubility | 24.6 mg/mL (EtOH)<br>49 mg/mL (DMSO) | 2 µg/mL (pH 7.4, free base)<br>47 mg/mL (DMSO, salt) | 1 µg/mL (cryst., pH 7]<br>9 mg/mL (cryst., EtOH) |
| *In vivo* activity | LH suppression in castr. male rats at 100 mg/kg | LH suppression in rats at 30 mg/kg (2HCl) | LH suppression in OVX-rats at 10 mg/kg |
| MW [g/mol] | 492 | 477 | 601 |
| Hum/rat pot. [nM] | $K_i$ = 6 / 3.8 | $IC_{50}$ = 12 / 71 | $IC_{50}$ = 21 / 24 |
| F (rat) [%] | 8-24 | 74 (2HCl) | 48-87 |

BAY-784 shows a favorable DMPK profile and is the first GnRH antagonist accompanied by a negative control compound

# GnRH-R Ant. Probe BAY-784

## Overall Profile




**BAY-784**

| Potency and Selectivity ($IC_{50}$) | |
|---|---|
| Hum* | **21 nM** |
| Rat* | **24 nM** |
| Monkey* | **35 nM** |
| Binding** | **27 nM** |
| MAPK14 / MAPK13 / CB1 ^ | **11 / 5 / 3.4 µM** |

^ 3 significant hits out of 122 off-targets tested

| Properties and PhysChem | |
|---|---|
| LogD @ pH 7.5 | **4.1** |
| LLE*** | **4.0** |
| $S_w$ pH 6.5 | **1 mg/L** |
| MWcorr | **601 g/mol** |
| TPSA | **114 Å²** |
| Rotatable Bonds | **7** |

| CYP Inhibition [µM] | | | | | |
|---|---|---|---|---|---|
| 1A2 | 2C8 | 2C9 | 2D6 | 3A4 | 3A4 (pre.) |
| **>10** | **1.0** | **>10** | **>10** | **>10** | **>10** |

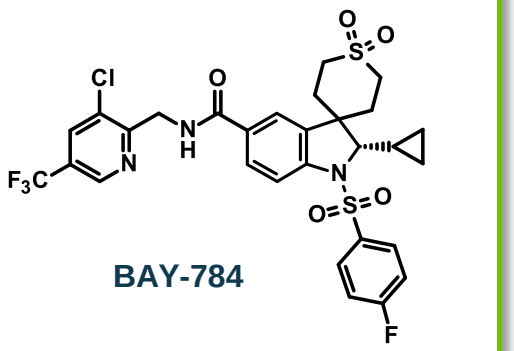
| CYP Induction (Hum Hep) | |
|---|---|
| NOEL CYP1A2 | NOEL CYP3A4 |
| **5000 ng/mL** | **1667 ng/mL** |

* mechan., biochem. IP1 assays, CHO cells stably expressing GnRH-R; Buserelin at ~$EC_{80}$
** human GnRH receptor binding assay (Tag Lite)
*** LLE = ligand-lipophilicity efficiency = p(IC50 hum) – clogD

**BAY-784 is a selective GnRH inhibitor with high potency *across* tested species**

# GnRH-R Ant. Probe BAY-784

## Overall Profile



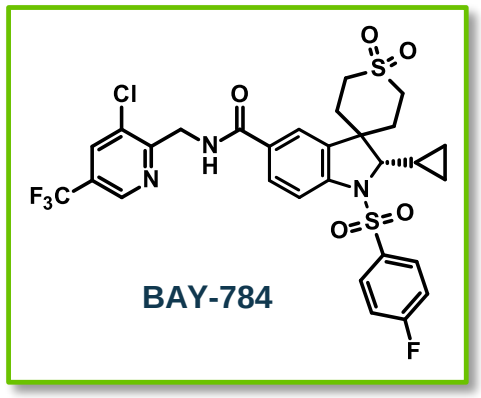

**BAY-784**

| **Pharmacokinetics** | | | | | |
|---|---|---|---|---|---|
| Caco-2 $P_{app}$ AB // efflux ratio | | | **12 nm/s // 10** | | |
| rat / hum Hep CL ($F_{max}$) | L/h/kg (%) | | **0.36-0.75 (82-91%) / 0.1-0.49 (63-93%)** | | |
| dog / cynom Hep CL ($F_{max}$) | L/h/kg (%) | | **0.73 (65%) / 0.71 (72%)** | | |
| $f_u$ (hum) | | | **1.4 %** | | |
| *In vivo* PK iv/po | $CL_b$ [L/h/kg] | $V_{ss}$ [L/kg] | $t_{1/2}$ [h] | po $AUC_{norm}$ [kg·h/L] | F [%] |
| Rat (male Wistar) | **0.54** | **10** | **13-17** | **0.8-1.4** | **48-87** |
| Dog (female Beagle) | **0.66** | **6.3** | **18** | **1.4** | **69** |
| Cynomolgus (female) | **1.0** | **3.6** | **7** | **0.2-0.5** | **16-40** |

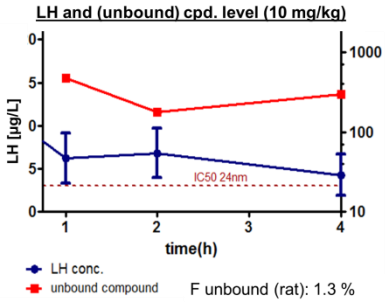
BAY-784 shows a preferable DMPK profile which makes the inhibitor suitable for *in vivo* studies (Phase 1 study performed)

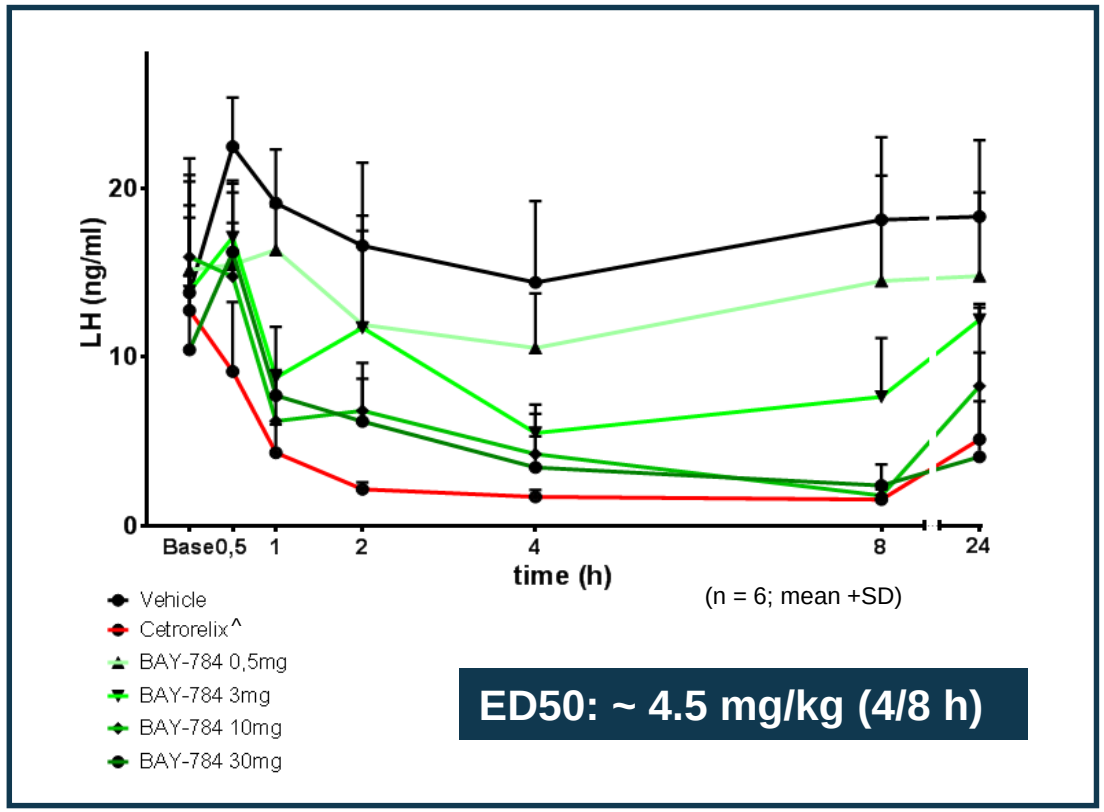
# GnRH-R Ant. Probe BAY-784

## In vivo Pharmacology Data: Female Rat




- Single p.o. application in OVX rats

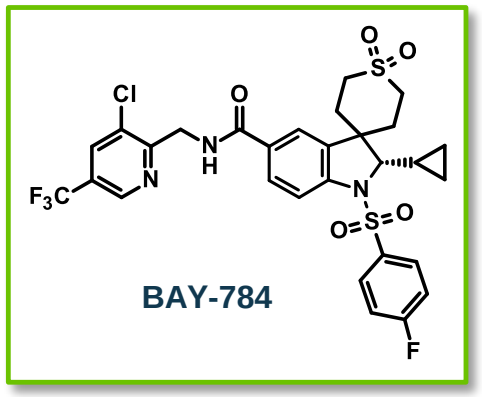


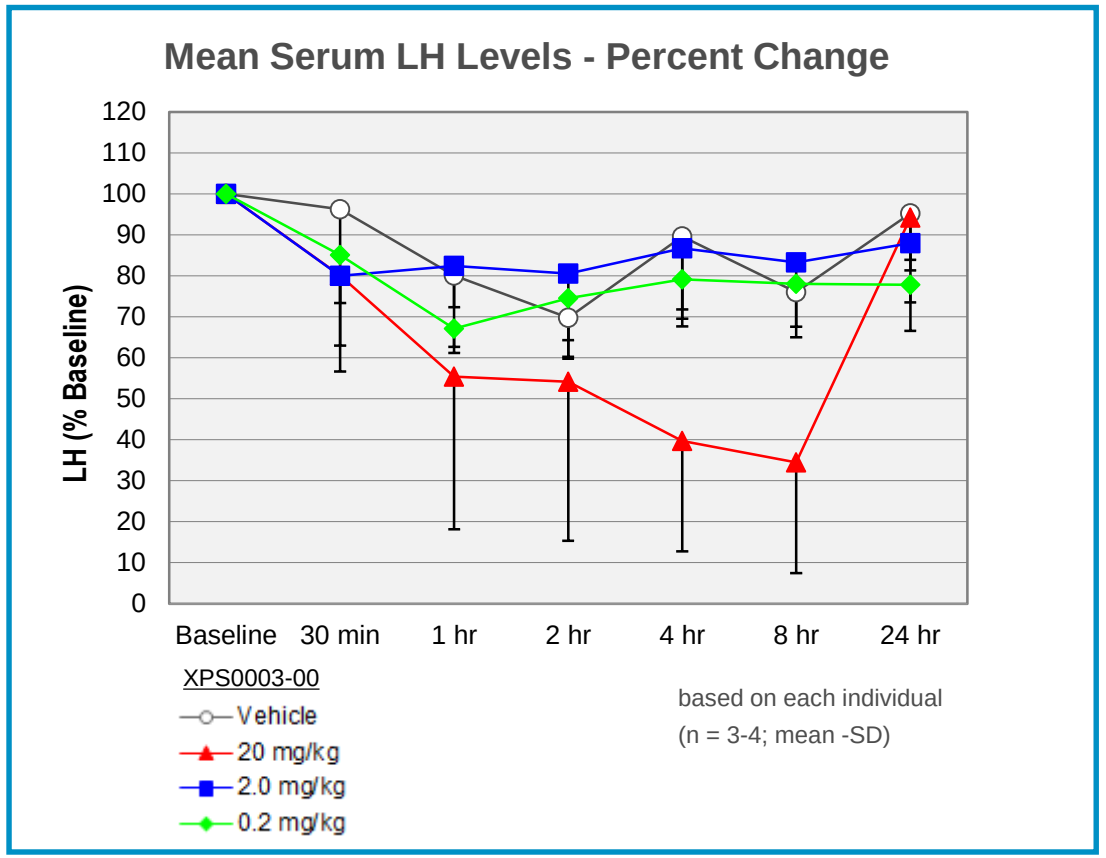

**BAY-784 suppressed the plasma LH concentration in OVX rats in a dose-dependent manner**

# GnRH-R Ant. Probe BAY-784

## In vivo Pharmacology Data: Male Monkey



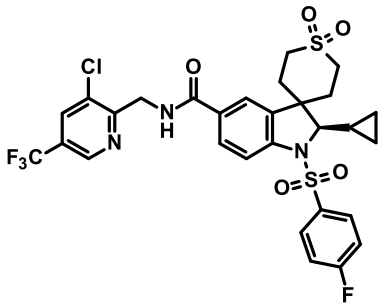

- Single p.o. application in ORX cynomolgus monkeys (8 weeks post surgery)
- Doses > 20 mg/kg not tested




**BAY-784 suppressed the plasma LH level at 20 mg/kg to approximately 40% (4/8 hours) of the initial concentration in ORX cynomolgous monkeys**

# GnRH-R Ant. Probe BAY-784

## Negative Control BAY-786




**BAY-786**

- **Molecular Properties**

| | |
|---|---|
| MW [g/mol] | 672 |
| MWcorr [g/mol] | 601 |
| TPSA [Å2] | 114 |
| Rotatable bonds | 7 |

- **PhysChem**

| | |
|---|---|
| Sol.$^{pH\ 6.5}$ [mg/L] | <0.1 |
| clog D (pH 7.5) | 2.5 |

sol. from DMSO; from solid: nd

- **Pharmacology**

| | |
|---|---|
| GnRH-R hum (Buserelin@$EC_{80}$) $IC_{50}$ | 2.4 µM* |

* arith. mean of 7 measurements; strongly dependent on residual levels of BAY-784 after enantiomeric separation

- **In vitro PK**

| | A-B [nm/s] | B-A [nm/s] | Ratio |
|---|---|---|---|
| CaCo2 | 7.8 | 300 | 39 |

# GnRH-R Ant. Probe BAY-784

## Summary / Conclusion

| Probe criteria | |
|---|---|
| **Inhibitor/agonist potency: goal is < 100 nM** (IC50, Kd) | **Surpasses criteria**; high potency in mechanistic, hum cell-based assay with $IC_{50}$ = 21 nM (superagonist Buserelin stimulation at $EC_{80}$) |
| **Selectivity within target family: goal is >30-fold** | **Surpasses criteria**; selectivity against a broad range of GPCR's (see Millipore GPCR profiler) |
| Selectivity outside target family: describe the off-targets (which may include both binding and functional data) | **Surpasses criteria**; clean LeadProfilingScreen; highest potency among 122 tested off-targets: CB1 ($IC_{50}$ = 3.4 µM) |
| **On target cell activity for cell-based targets: goal is < 1 micromolar** IC50/EC50 | **Surpasses criteria**; high potency in hum cell-based assay with $IC_{50}$ = 21 nM (superagonist Buserelin stimulion at $EC_{80}$) |
| On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture | **Surpasses criteria**; suitable pharmacokinetic profile for in vivo studies; in vivo efficacy in LH suppression experiments in female rats and male monkeys shown |
| Neg ctrl: *in vitro* potency –> 100 times less; Cell activity –> 100 times less potent than the probe | **Surpasses criteria**; BAY-786 with at least 114-fold lower in vitro potency ($IC_{50}$ = 2.4 µM) |

**We ask for acceptance of GnRH-R antagonist BAY-784 as *in vitro* / *in vivo* chemical probe, accompanied by BAY-786 as negative control**

# GnRH-R Ant. Probe BAY-784

## Acknowledgement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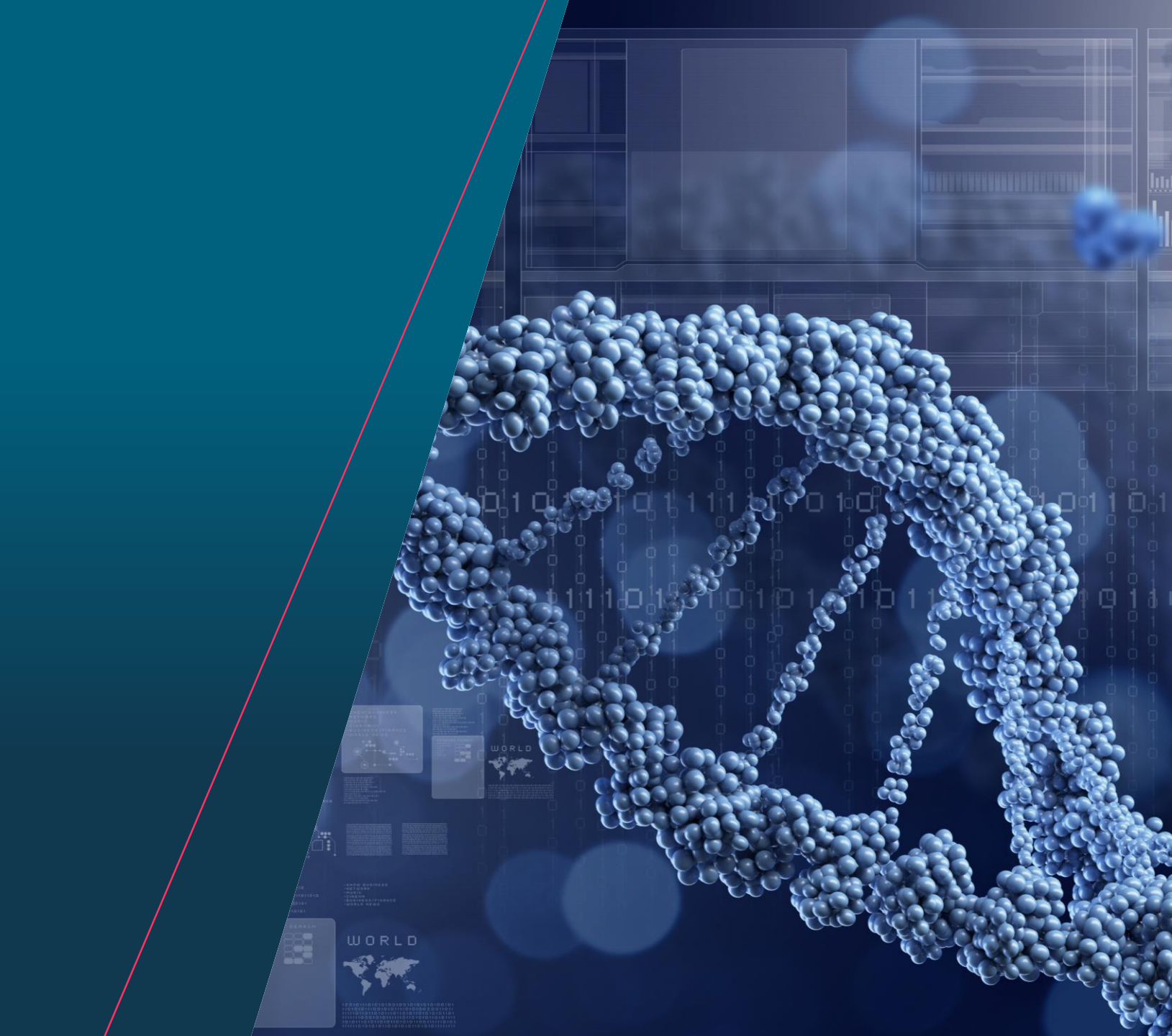
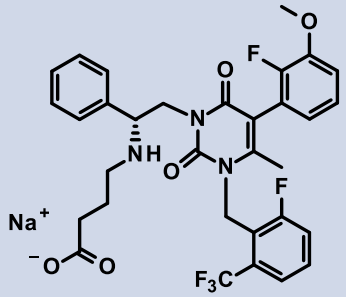
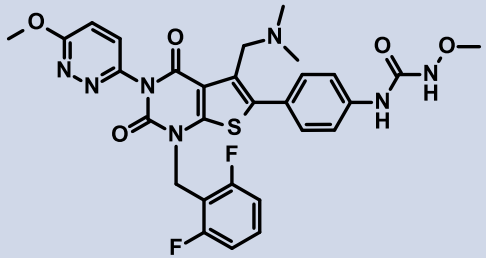
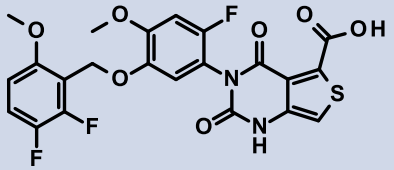
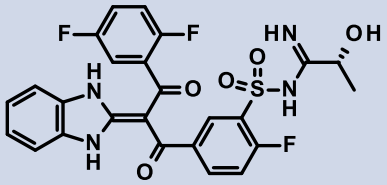
and their teams

# *Thank You*



# GnRH-R Ant. Probe BAY-784

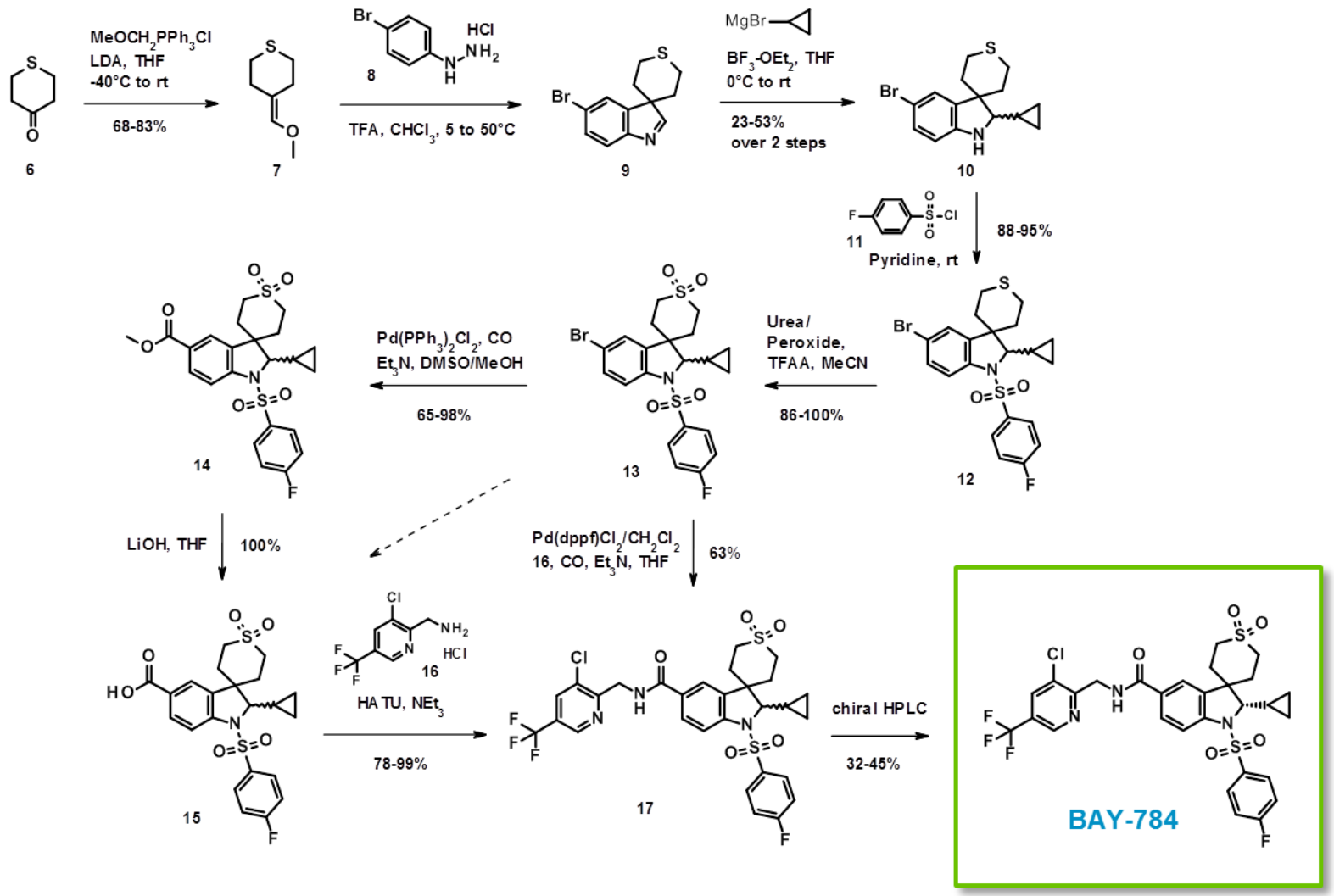
## Oral GnRH Antagonists in Development

| Company / Drug Name | AbbVie/Neurocrine Biosc.: **Elagolix** (NBI-56418 Na) | Takeda/Myovant: **Relugolix** (TAK-385) | Kissei/ObsEva: **Linzagolix** (OBE-2109) | Astellas: **ASP-1707** |
|---|---|---|---|---|
| Structure |  |  |  |  |
| Active Indications | endometriosis; uterine fibroids | endometriosis; uterine fibroids; prostate tumor | endometriosis; uterine fibroids | endometriosis; rheumatoid arthritis |
| Highest Devel. Status | Ph III | Ph III | Ph III | Ph II |
| Add. Information | uterine fibroids: 6 (+6) months studies; elagolix alone or with add-back therapy in curr. Ph III | uterine fibroids: 40 mg once daily for 12-48 weeks (with hormonal add-back therapy) in curr. Ph III | | |
| MW [g/mol] | 654 | 624 | 508 | 545 |
| $IC_{50}$ rat [nM] | 3120 | 9800 | not avail. | not avail. |

BAY-784 shows a favorable DMPK profile and a high potency across species including rats

# GnRH-R Ant. Probe BAY-784

## Synthesis of BAY-784



# GnRH-R Ant. Probe BAY-784

## GPCR Panel (25 GPCR's, Millipore)

### Agonist Data (Percentage Activation Normalized to $E_{max}$ Control)

| GPCR Target | 08_2012_02 @ 12.5µM | | |
|---|---|---|---|
| | n1 | n2 | Avg. |
| 5-HT1A | -5.6 | 6.6 | 0.5 |
| 5-HT2B | 3.1 | 3.0 | 3.1 |
| 5-HT6 | 13.0 | 14.2 | 13.6 |
| A2B | 5.4 | 4.8 | 5.1 |
| A3 | 1.5 | 1.4 | 1.4 |
| ADRA1A | 1.7 | 1.7 | 1.7 |
| ADRA2A | 1.2 | 1.3 | 1.3 |
| ADRB1 | 1.3 | 1.5 | 1.4 |
| ADRB2 | 1.4 | 2.5 | 2.0 |
| CB1 | 2.3 | 3.1 | 2.7 |
| D1 | 6.8 | 8.0 | 7.4 |
| D2 | 7.9 | 8.5 | 8.2 |
| EP3 | 1.0 | 1.1 | 1.1 |
| H1 | 5.2 | 6.1 | 5.6 |
| H2 | 2.1 | 0.9 | 1.5 |
| H3 | -0.3 | 0.3 | 0.0 |
| M1 | 1.0 | 1.1 | 1.0 |
| M4 | 0.6 | 0.7 | 0.7 |
| MC4 | -0.2 | 0.9 | 0.4 |
| Motilin | 1.3 | 1.3 | 1.3 |
| NK1 | 1.4 | 0.2 | 0.8 |

| GPCR Target | 08_2012_02 @ 12.5µM | | |
|---|---|---|---|
| | n1 | n2 | Avg. |
| OPRK1 | 14.8 | 8.4 | 11.6 |
| OPRM1 | 2.2 | 3.1 | 2.6 |
| P2RY2 | -1.1 | -1.2 | -1.2 |
| SST4 | 1.8 | -0.6 | 0.6 |

BAY-784 = 08_2012_02

### Antagonist Data (Percentage Inhibition)

| GPCR Target | 08_2012_02 @ 10µM | | |
|---|---|---|---|
| | n1 | n2 | Avg. |
| 5-HT1A | -4.8 | 1.1 | -1.9 |
| 5-HT2B | 12.9 | 5.3 | 9.1 |
| 5-HT6 | 10.0 | 3.7 | 6.8 |
| A2B | 12.8 | 13.1 | 13.0 |
| A3 | 14.0 | 5.1 | 9.5 |
| ADRA1A | 8.9 | 1.2 | 5.1 |
| ADRA2A | -9.7 | -11.4 | -10.5 |
| ADRB1 | 5.9 | 2.8 | 4.3 |
| ADRB2 | 1.8 | 5.4 | 3.6 |
| CB1 | 10.3 | 6.2 | 8.2 |
| D1 | 8.6 | 12.0 | 10.3 |
| D2 | 25.7 | 24.9 | 25.3 |
| EP3 | 13.1 | 5.7 | 9.4 |
| H1 | 8.4 | -3.2 | 2.6 |
| H2 | 8.7 | 12.5 | 10.6 |
| H3 | -10.8 | 5.6 | -2.6 |
| M1 | 3.7 | -1.0 | 1.4 |
| M4 | 9.1 | 13.1 | 11.1 |
| MC4 | -3.3 | -1.3 | -2.3 |
| Motilin | 21.1 | 8.8 | 15.0 |
| NK1 | 4.6 | 2.5 | 3.6 |
| OPRK1 | -45.0 | -28.8 | -36.9 |
| OPRM1 | 7.0 | 21.8 | 14.4 |
| P2Y2 | -9.3 | -14.9 | -12.1 |
| SST4 | 10.3 | 16.1 | 13.2 |

# GnRH-R Ant. Probe BAY-784

## LeadprofilingScreen Data (Ricerca)

| Cat # | Assay Name | Batch* | Spec. | Rep. | Conc. | % Inh. | IC50* |
|---|---|---|---|---|---|---|---|
| **Compound: CHH54-2012, PT #: 1163668** | | | | | | | |
| 107000 | Aldose Reductase | 319779 | rat | 2 | 10 µM | 0 | |
| 107710 | ATPase, $Na^+/K^+$, Heart, Pig | 319782 | pig | 2 | 10 µM | 4 | |
| 126000 | Beta-Lactamase | 319809 | ba | 2 | 10 µM | -2 | |
| 112020 | Carbonic Anhydrase II | 319784 | hum | 2 | 10 µM | -5 | |
| 104010 | Cholinesterase, Acetyl, ACES | 319764 | hum | 2 | 10 µM | 56 | |
| 116020 | Cyclooxygenase COX-1 | 319816 | hum | 2 | 10 µM | -12 | |
| 118010 | Cyclooxygenase COX-2 | 319818 | hum | 2 | 10 µM | 4 | |
| 118050 | CYP450, 1A2 | 319747 | hum | 2 | 10 µM | -2 | |
| 118070 | CYP450, 2C19 | 319749 | hum | 2 | 10 µM | 36 | |
| 118060 | CYP450, 2C9 | 319748 | hum | 2 | 10 µM | 32 | |
| 118080 | CYP450, 2D6 | 319750 | hum | 2 | 10 µM | 4 | |
| 118090 | CYP450, 3A4 | 319751 | hum | 2 | 10 µM | 38 | |
| 124010 | HMG-CoA Reductase | 319808 | hum | 2 | 10 µM | 12 | |
| 132000 | Leukotriene $LTC_4$ Synthase | 319810 | gp | 2 | 10 µM | -9 | |
| 138000 | Lipoxygenase 15-LO | 319811 | rabbit | 2 | 10 µM | 11 | |
| 140010 | Monoamine Oxidase MAO-A | 319665 | hum | 2 | 10 µM | 3 | |
| 144000 | Nitric Oxide Synthase, Inducible (iNOS) | 320013 | mouse | 2 | 10 µM | 6 | |
| 142000 | Nitric Oxide Synthase, Neuronal (nNOS) | 320012 | rat | 2 | 10 µM | 2 | |
| 107300 | Peptidase, Angiotensin Converting Enzyme | 319781 | rabbit | 2 | 10 µM | 0 | |
| 163000 | Peptidase, CASP1 (Caspase 1) | 319813 | hum | 2 | 10 µM | 8 | |
| 112510 | Peptidase, CTSG (Cathepsin G) | 319908 | hum | 2 | 10 µM | 0 | |
| 166010 | Peptidase, ELA2 (Neutrophil Elastase 2) | 319757 | hum | 2 | 10 µM | 4 | |
| 114110 | Peptidase, Matrix Metalloproteinase-1 (MMP-1) | 320014 | hum | 2 | 10 µM | -4 | |
| 114910 | Peptidase, Matrix Metalloproteinase-9 (MMP-9) | 320016 | hum | 2 | 10 µM | 3 | |
| 152000 | Phosphodiesterase PDE3 | 319828 | hum | 2 | 10 µM | 9 | |
| 154000 | Phosphodiesterase PDE4 | 319804 | hum | 2 | 10 µM | 2 | |
| 156000 | Phosphodiesterase PDE5 | 319829 | hum | 2 | 10 µM | -5 | |
| 171120 | Protein Serine/Threonine Kinase, MAPK1 (ERK2) | 319950 | hum | 2 | 10 µM | 53 | |
| 176610 | Protein Serine/Threonine Kinase, MAPK14 (p38α) | 319958 | hum | 2 | 10 µM | 63 | |
| 171000 | Protein Serine/Threonine Kinase, MAPK3 (ERK1) | 319949 | hum | 2 | 10 µM | 71 | |
| 180010 | Protein Serine/Threonine Kinase, PRKCA (PKCα) | 319967 | hum | 2 | 10 µM | 16 | |

| Cat # | Assay Name | Batch* | Spec. | Rep. | Conc. | % Inh. | IC50* |
|---|---|---|---|---|---|---|---|
| 188020 | Protein Serine/Threonine Phosphatase, PPP3CA (Calcineurin, PP2B) | 320030 | hum | 2 | 10 µM | -8 | |
| 170020 | Protein Tyrosine Kinase, EGF Receptor | 319766 | hum | 2 | 10 µM | -3 | |
| 174020 | Protein Tyrosine Kinase, ERBB2 (HER2) | 319964 | hum | 2 | 10 µM | 6 | |
| 172020 | Protein Tyrosine Kinase, Fyn | 319773 | hum | 2 | 10 µM | 5 | |
| 176020 | Protein Tyrosine Kinase, LCK | 319785 | hum | 2 | 10 µM | 0 | |
| 190010 | Protein Tyrosine Phosphatase, PTPRC (CD45) | 319895 | hum | 2 | 10 µM | -5 | |
| 194020 | Thromboxane Synthase | 319812 | hum | 2 | 10 µM | 22 | |
| 200510 | Adenosine $A_1$ | 319717 | hum | 2 | 10 µM | 15 | |
| 200610 | Adenosine $A_{2A}$ | 319668 | hum | 2 | 10 µM | 0 | |
| 200720 | Adenosine $A_3$ | 319695 | hum | 2 | 10 µM | 9 | |
| 203100 | Adrenergic $\alpha_{1A}$ | 319719 | rat | 2 | 10 µM | 7 | |
| 203200 | Adrenergic $\alpha_{1B}$ | 319720 | rat | 2 | 10 µM | 2 | |
| 203400 | Adrenergic $\alpha_{1D}$ | 319721 | hum | 2 | 10 µM | 18 | |
| 203620 | Adrenergic $\alpha_{2A}$ | 319669 | hum | 2 | 10 µM | -1 | |
| 203710 | Adrenergic $\alpha_{2B}$ | 319670 | hum | 2 | 10 µM | 4 | |
| 203800 | Adrenergic $\alpha_{2C}$ | 319671 | hum | 2 | 10 µM | 3 | |
| 204010 | Adrenergic $\beta_1$ | 319714 | hum | 2 | 10 µM | 7 | |
| 204110 | Adrenergic $\beta_2$ | 319715 | hum | 2 | 10 µM | -4 | |
| 204200 | Adrenergic $\beta_3$ | 319716 | hum | 2 | 10 µM | 0 | |
| 285010 | Androgen (Testosterone) AR | 319699 | rat | 2 | 10 µM | 16 | |
| 210120 | Angiotensin $AT_2$ | 319909 | hum | 2 | 10 µM | -5 | |
| 211000 | Atrial Natriuretic Factor (ANF) | 319903 | gp | 2 | 10 µM | 2 | |
| 212620 | Bradykinin $B_2$ | 319726 | hum | 2 | 10 µM | 9 | |
| 213610 | Calcitonin | 320010 | hum | 2 | 10 µM | -22 | |
| 214510 | Calcium Channel L-Type, Benzothiazepine | 319727 | rat | 2 | 10 µM | 29 | |
| 214600 | Calcium Channel L-Type, Dihydropyridine | 319675 | rat | 2 | 10 µM | 26 | |
| 215000 | Calcium Channel L-Type, Phenylalkylamine | 320009 | rat | 2 | 10 µM | 47 | |
| 217030 | Cannabinoid $CB_1$ | 319676 | hum | 2 | 10 µM | 73 | |
| 217550 | Chemokine CCR2B | 320006 | hum | 2 | 10 µM | -1 | |
| 217660 | Chemokine CCR4 | 320007 | hum | 2 | 10 µM | -20 | |
| 217720 | Chemokine CCR5 | 320008 | hum | 2 | 10 µM | -1 | |
| 244500 | Chemokine CXCR2 (IL-$8R_B$) | 319879 | hum | 2 | 10 µM | 14 | |
| 218020 | Cholecystokinin $CCK_1$ ($CCK_A$) | 319772 | hum | 2 | 10 µM | -3 | |
| 219500 | Dopamine $D_1$ | 319677 | hum | 2 | 10 µM | 6 | |
| 219600 | Dopamine $D_{2L}$ | 319854 | hum | 2 | 10 µM | 4 | |

# GnRH-R Ant. Probe BAY-784

## LeadprofilingScreen Data (Ricerca)

| Cat # | Assay Name | Batch* | Spec. | Rep. | Conc. | % Inh. | $IC_{50}$* |
|---|---|---|---|---|---|---|---|
| 219800 | Dopamine $D_3$ | 319679 | hum | 2 | 10 µM | 17 | |
| 219900 | Dopamine $D_{4.2}$ | 319680 | hum | 2 | 10 µM | 11 | |
| 224010 | Endothelin $ET_A$ | 319792 | hum | 2 | 10 µM | 16 | |
| 226010 | Estrogen ERα | 319733 | hum | 2 | 10 µM | 21 | |
| 226810 | $GABA_A$, Chloride Channel, TBOB | 319722 | rat | 2 | 10 µM | 34 | |
| 226600 | $GABA_A$, Flunitrazepam, Central | 319681 | rat | 2 | 10 µM | -1 | |
| 226500 | $GABA_A$, Muscimol, Central | 319682 | rat | 2 | 10 µM | 7 | |
| 232030 | Glucocorticoid | 319770 | hum | 2 | 10 µM | 13 | |
| 232600 | Glutamate, AMPA | 319900 | rat | 2 | 10 µM | 2 | |
| 232700 | Glutamate, Kainate | 319705 | rat | 2 | 10 µM | 1 | |
| 232810 | Glutamate, NMDA, Agonism | 319706 | rat | 2 | 10 µM | -13 | |
| 233000 | Glutamate, NMDA, Phencyclidine | 319708 | rat | 2 | 10 µM | -4 | |
| 239000 | Glycine, Strychnine-Sensitive | 319876 | rat | 2 | 10 µM | 4 | |
| 239610 | Histamine $H_1$ | 319683 | hum | 2 | 10 µM | 9 | |
| 239710 | Histamine $H_2$ | 319696 | hum | 2 | 10 µM | 1 | |
| 241000 | Imidazoline $I_2$, Central | 319684 | rat | 2 | 10 µM | 10 | |
| 243000 | Insulin | 320004 | rat | 2 | 10 µM | -1 | |
| 250460 | Leukotriene, Cysteinyl $CysLT_1$ | 319728 | hum | 2 | 10 µM | 4 | |
| 251300 | Melanocortin $MC_3$ | 320001 | hum | 2 | 10 µM | -7 | |
| 251350 | Melanocortin $MC_4$ | 319880 | hum | 2 | 10 µM | 12 | |
| 251400 | Melanocortin $MC_5$ | 320003 | hum | 2 | 10 µM | 27 | |
| 252610 | Muscarinic $M_1$ | 319685 | hum | 2 | 10 µM | 5 | |
| 252710 | Muscarinic $M_2$ | 319686 | hum | 2 | 10 µM | -3 | |
| 252810 | Muscarinic $M_3$ | 319687 | hum | 2 | 10 µM | 1 | |
| 252910 | Muscarinic $M_4$ | 320048 | hum | 2 | 10 µM | 2 | |
| 253010 | Muscarinic $M_5$ | 320049 | hum | 2 | 10 µM | 0 | |
| 257010 | Neuropeptide Y $Y_1$ | 319797 | hum | 2 | 10 µM | 17 | |
| 257110 | Neuropeptide Y $Y_2$ | 319796 | hum | 2 | 10 µM | 2 | |
| 258590 | Nicotinic Acetylcholine | 319703 | hum | 2 | 10 µM | -15 | |
| 260130 | Opiate $\delta_1$ (OP1, DOP) | 319860 | hum | 2 | 10 µM | 5 | |
| 260210 | Opiate κ(OP2, KOP) | 319861 | hum | 2 | 10 µM | 7 | |
| 260410 | Opiate µ(OP3, MOP) | 319688 | hum | 2 | 10 µM | 16 | |
| 264500 | Phorbol Ester | 319689 | mouse | 2 | 10 µM | 1 | |
| 265010 | Platelet Activating Factor (PAF) | 319758 | hum | 2 | 10 µM | 6 | |
| 265200 | Platelet-Derived Growth Factor (PDGF) | 319694 | mouse | 2 | 10 µM | -4 | |

| Cat # | Assay Name | Batch* | Spec. | Rep. | Conc. | % Inh. | $IC_{50}$* |
|---|---|---|---|---|---|---|---|
| 265600 | Potassium Channel [$K_{ATP}$] | 319709 | ham | 2 | 10 µM | 0 | |
| 268000 | Progesterone | 319798 | bov | 2 | 10 µM | 0 | |
| 268700 | Purinergic $P_{2X}$ | 319697 | rabbit | 2 | 10 µM | -17 | |
| 271110 | Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$ | 319807 | hum | 2 | 10 µM | 0 | |
| 271200 | Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1B}$ | 319835 | rat | 2 | 10 µM | -24 | |
| 271700 | Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$ | 319692 | hum | 2 | 10 µM | 4 | |
| 271800 | Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$ | 319730 | hum | 2 | 10 µM | 9 | |
| 271910 | Serotonin (5-Hydroxytryptamine) $5\text{-}HT_3$ | 319778 | hum | 2 | 10 µM | 19 | |
| 272000 | Serotonin (5-Hydroxytryptamine) $5\text{-}HT_4$ | 319825 | gp | 2 | 10 µM | 22 | |
| 272200 | Serotonin (5-Hydroxytryptamine) $5\text{-}HT_6$ | 319911 | hum | 2 | 10 µM | 14 | |
| 278110 | Sigma $\sigma_1$ | 319711 | hum | 2 | 10 µM | 15 | |
| 278200 | Sigma $\sigma_2$ | 319913 | rat | 2 | 10 µM | 20 | |
| 255520 | Tachykinin $NK_1$ | 319795 | hum | 2 | 10 µM | 26 | |
| 255600 | Tachykinin $NK_2$ | 319925 | hum | 2 | 10 µM | 7 | |
| 202000 | Transporter, Adenosine | 319718 | gp | 2 | 10 µM | 25 | |
| 220320 | Transporter, Dopamine (DAT) | 319673 | hum | 2 | 10 µM | 25 | |
| 204410 | Transporter, Norepinephrine (NET) | 319672 | hum | 2 | 10 µM | 14 | |
| 274030 | Transporter, Serotonin (5-Hydroxytryptamine) (SERT) | 319674 | hum | 2 | 10 µM | 10 | |
| 286510 | Tumor Necrosis Factor (TNF), Non-Selective | 319983 | hum | 2 | 10 µM | 22 | |
| 287010 | Vasoactive Intestinal Peptide $VIP_1$ | 319902 | hum | 2 | 10 µM | 2 | |
| 287530 | Vasopressin $V_{1A}$ | 319822 | hum | 2 | 10 µM | -11 | |

# GnRH-R Ant. Probe BAY-784

## Relevant IC50 Data (Ricerca)

| Cat # | Assay Name | Species | Conc. | % Inh. | $IC_{50}$* | $K_i$ | $n_H$ |
|---|---|---|---|---|---|---|---|
| 171000 | Protein Serine/Threonine Kinase, MAPK3 (ERK1) | hum | 10 µM | 87 | 4.87 µM | | |
| 176610 | Protein Serine/Threonine Kinase, MAPK14 (p38α) | hum | 10 µM | 50 | 10.7 µM | | |
| 217030 | Cannabinoid $CB_1$ | hum | 3 µM | 52 | 3.44 µM | 2.57 µM | 0.91 |

# GnRH-R Ant. Probe BAY-784

## Mechanistic Assay Description (Human Receptor)

**Human, hGnRH-R IC50 determination (one day protocol)**

Agonist binding to the hGnRH-R results in the activation of phospholipase C leading to the production of inositol-3-phosphate (IP3) and the subsequent release of intracellular Ca++.

Termination of second messenger signaling is achieved through the conversion of IP3 to myoinositol [via inositol-2- (IP2) and inositol-1-phosphate (IP1)], a process, which can be blocked at the IP1-level in the presence of LiCl. The resulting accumulation of cellular IP1 is used in a competitive immunoassay in which IP1 competes with a fluorescent IP1-tracer (IP1-d2) for the binding to a terbium labelled anti-IP1 antibody (CisBio International; HTRF IPOne Assay).

Maximum signal resulting from fluorescence resonance energy transfer (FRET) between the detection reagents is obtained in the absence of cellular IP1. Any decrease in FRET signal is indicative of hGnRH-R activation whereas antagonist activity results in signal increase once again. FRET signal quantification is achieved with the help of an appropriate plate reader (PheraStar; RubyStar; ViewLux). Following excitation at 340 nm any reduction of FRET induced emissions at 520 nm is indicative of agonist induced IP1 production. In addition, a second FRET signal at 495 nm, originating from the Tb-labelled Anti IP1 antibody, is used for well internal referencing (Well-Ratio; defined as 520nm/495nm*10000).

Typically, the reaction volume was 5 µl in 384 well plates. Three microliters of the cell suspension containing 3333 cells/µl (10 000 cells/well) in Ham´s F12 medium were added to all wells of the ready to use test plate. Following a 20 minute preincubation at RT two microliters of a 2.5XEC80 agonist solution of either LHRH (X.XXE-ZM; Source = ;Stock: 80µM in 10mM in Tris/HCl 0.01% BSA, stored at -20°C) or Buserelin (Y.YY E-ZM; USbiological, #B8995, Stock: 0,1mg/ml in Tris-Cl; 8,07E-05M, stored at -20°C) prepared fresh in stimulation buffer (10mM Hepes pH 7.4, 1mM CaCl2, 0.5mM MgCl2, 4.2mM KCl, 146mM NaCl, 5.5mM alpha-D-Glucose, 0.05% BSA, 150mM) was added to the test compound and positive control wells (low controls [C(0)]). The negative control wells (high controls, [C(i)]) received stimulation buffer only. Following that, the plate was incubated for another 60 minutes at 37°C in the presence of a 1XEC80 concentration of agonist (LHRH: N.NNE-NNM; Buserelin: n.nnE-nnM). The reaction was stopped by the addition of 2 µl of lysisbuffer containing a 1:38 dilution of the Tb-cryptate labelled anti-IP1 antibody stock prepared according the manufacturers protocol. Another 60 minutes later the cell lysate containing plate was transferred to TR-FRET compatible reader in order to quantify the results.