

Donated Chemical Probe

GnRH-R Antagonist
Probe BAY-784

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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

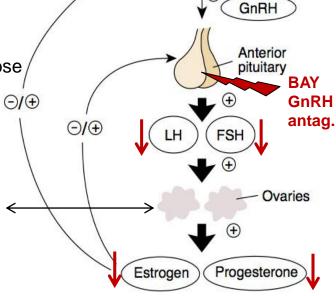
// GnRH superagonists (to be injected) & antagonists block this process → leading to impaired ovarian steroidogenesis

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

Male HPG Axis

LH and FSH
Chemical castration

Androgens



Female HPG Axis

Hypothalamus

HPG axis / impaired testicular androgen production and spermatogenesis

GnRH antag. – similar effect in male

adapted from Friedman et al., 1990



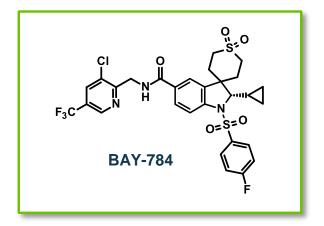
Two Commercially Available SMOL GnRH Antagonists with Activity at Rodent Receptor

Company / Identifier	Pfizer: AG 045572	Wyeth: WAY-207024	Bayer: BAY-784
Structure			F ₃ C
CAS No.	[263847-55-8]	2HCI: [872002-73-8]	[1631164-24-3]
Solubility	24.6 mg/mL (EtOH) 49 mg/mL (DMSO)	2 μg/mL (pH 7.4, free base) 47 mg/mL (DMSO, salt)	1 μg/mL (cryst., pH 7] 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 (2HCI)	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 (2HCI)	48-87

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



Overall Profile



Potency and Selectivity (IC ₅₀)	
Hum*	21 nM
Rat*	24 nM
Monkey*	35 nM
Binding**	27 nM
MAPK14 / MAPK13 / CB1 ^	11 / 5 / 3.4 μΜ

LogD @ pH 7.5	4.1
LLE***	4.0
S _w pH 6.5	1 mg/L
MWcorr	601 g/mol
TPSA	114 Ų
Rotatable Bonds	7

Properties and PhysChem

CYP	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	

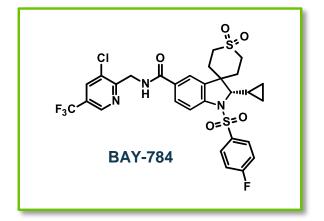
^{*} mechan., biochem. IP1 assays, CHO cells stably expressing GnRH-R; Buserelin at ~EC₈₀

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

^{**} human GnRH receptor binding assay (Tag Lite)

^{***} LLE = ligand-lipophilicity efficiency = p(IC50 hum) - clogD



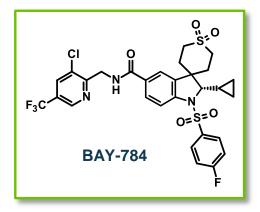


Pharmacokinetics					
Caco-2 P _{app} AB // efflux ratio			12 nm/s // 10		
rat / hum Hep CL (F _{max}) L/h/kg (%)		0.36-0.75	0.36-0.75 (82-91%) / 0.1-0.49 (63-93%)		
dog / cynom Hep CL (F _{max})	og / cynom Hep CL (F _{max}) L/h/kg (%)		0.73 (65%) / 0.71 (72%)		
f _u (hum)				1.4 %	
In vivo PK iv/po	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

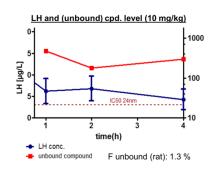
BAY-784 shows a preferable DMPK profile which makes the inhibitor suitable for *in vivo* studies (Phase 1 study performed)

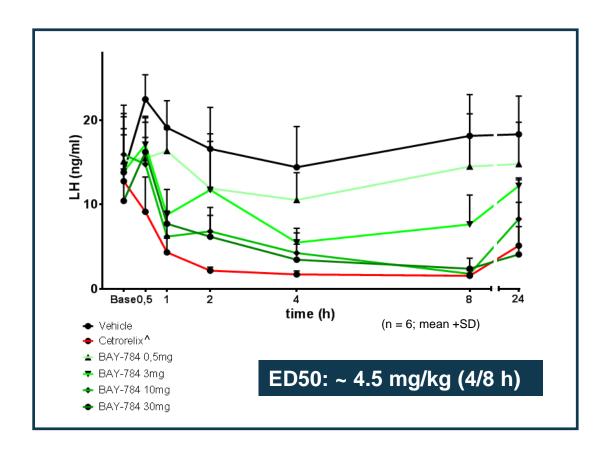


In vivo Pharmacology Data: Female Rat



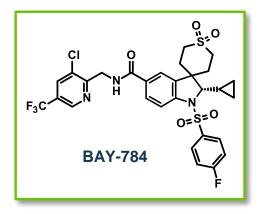
Single p.o. application in OVX rats



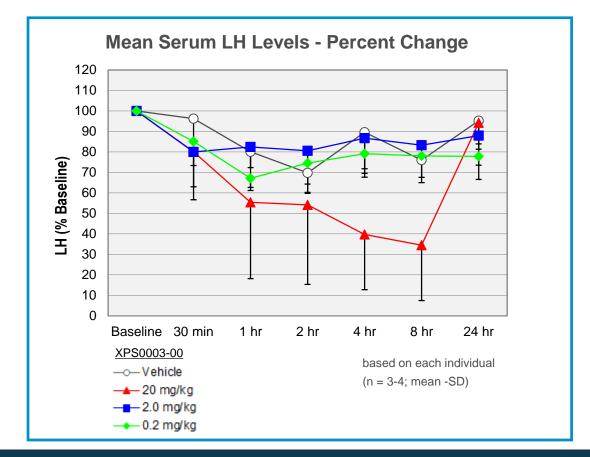


BAY-784 suppressed the plasma LH concentration in OVX rats in a dose-dependent manner

In vivo Pharmacology Data: Male Monkey



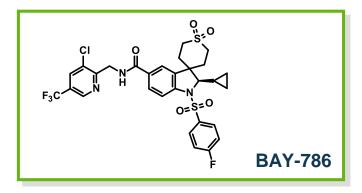
- Single p.o. application in ORX cynomolgus monkeys (8 weeks post surgery)
- Doses > 20 mg/kg not tested



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



Negative Control 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*
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^{*} arith. mean of 7 measurements; strongly dependent on residual levels of BAY-784 after enantiomeric separation

In vitro PK

CaCo2	A-B [nm/s]	B-A [nm/s]	Ratio
CaCOZ	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 μ M)
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: <i>in vitro</i> 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 μ M)

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

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Jan-Bernd Lenfers

Hendrik Lück

Kathrin Machens

Tobias Marquardt

Martin Merz Uwe Muenster

Hartwig Müller Peter Muhn

Marlies Nern

Katrin Nowak-Reppel

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Wolfgang Schwede

Beate Seibert

Andreas Steinmeyer Antonius Ter Laak Ildikó Terebesi Claudia Vogt Gabriele Winter Hans-Joachim Zentel

Thomas Zollner Ulrich Zügel

and their teams



Thank You





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	Na ⁺ -OOO F3C		O O O O O O O O O O O O O O O O O O O	F—F HN OH O O SS-N H O F F	
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



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				
Н3	-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_	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					
Н3	-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					



LeadprofilingScreen Data (Ricerca)

The compound Chils	Cat#	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC ₅₀ *	Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC ₅₀ *
10710 APPase, Na*K*, Heart, Pig 319782 pig 2 10 µM 4 174020 Protein Tyrosine Kinase, ERB82 (HER2) 31994 hum 2 10 µM 6 122000 Carbonic Arhydrase il 319606 ha 2 10 µM -2 172020 Protein Tyrosine Kinase, ERB82 (HER2) 31994 hum 2 10 µM 6 110200 Carbonic Arhydrase il 319764 hum 2 10 µM -5 176020 Protein Tyrosine Kinase, ERB82 (HER2) 31994 hum 2 10 µM 6 110200 Carbonic Arhydrase il 319764 hum 2 10 µM -5 176020 Protein Tyrosine Kinase, ERB82 (HER2) 319948 hum 2 10 µM 6 110200 Carbonic Arhydrase il 319764 hum 2 10 µM -12 19400 Protein Tyrosine Kinase, ERB82 (HER2) 319948 hum 2 10 µM 6 110200 Cyrlosoygenase COX-1 319816 hum 2 10 µM 4 200516 Adenosine A; 319717 hum 2 10 µM 6 118050 CYP450, 1A2 319717 hum 2 10 µM 36 200720 Adreneric ava 319608 hum 2 10 µM 7 118060 CYP450, 2C19 319749 hum 2 10 µM 36 200720 Adreneric ava 319719 rat 2 10 µM 7 118060 CYP450, 2C19 319751 hum 2 10 µM 38 20340 Adreneric ava 319719 rat 2 10 µM 6 118060 CYP450, 3A4 319751 hum 2 10 µM 38 20340 Adreneric ava 319721 hum 2 10 µM 18 124010 Humo-cora Exprise Arbonic Archydrase in Arb	Compo	ound: CHH54-2012, PT #: 1163668							188020		320030	hum	2	10 µM	-8	
12000 Beta-Laciamise 31980 ba 2 10 μM -5 17000 Protein Tyrosine Knase, Fyn 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319775 hum 2 10 μM -5 17000 Protein Tyrosine Knase, LCK 319977 hum 2 10 μM -5 17000 Protein Tyrosine Kna	107000	Aldose Reductase	319779	rat	2	10 µM	0		170020	Protein Tyrosine Kinase, EGF Receptor	319766	hum	2	10 µM	-3	
1700 Carbonic Anhydrase 319764 Num 2 10 µM -5 176020 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 176020 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 176020 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 319885 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK 3198785 Num 2 10 µM -5 180010 Protein Tyrosine Kinase, LCK	107710	ATPase, Na+/K+, Heart, Pig	319782	pig	2	10 µM	4		174020	Protein Tyrosine Kinase, ERBB2 (HER2)	319964	hum	2	10 µM	6	
104010 Cholinesterase, Acekyl, ACES 319764 hum 2 10 µM 56 190010 Protein Tyrosine Phosphatase, PTPRC (CD45) 319895 hum 2 10 µM 4-15 118002 Cyclooxygenase COX-1 319816 hum 2 10 µM 4-2 200610 Adenosine A₁ 319717 hum 2 10 µM 4-15 118002 CYP450, IA2 319747 hum 2 10 µM 2-2 200610 Adenosine A₃ 319668 hum 2 10 µM 4-15 118002 CYP450, IA2 319747 hum 2 10 µM 36 200720 Adenosine A₃ 319668 hum 2 10 µM 4-15 118002 CYP450, ZOF9 319748 hum 2 10 µM 36 200720 Adenosine A₃ 319668 hum 2 10 µM 3-15 118002 CYP450, ZOF9 319748 hum 2 10 µM 34 203300 Adrenergic a₃ 319720 rat 2 10 µM 2-15 118002 CYP450, ZOF9 319750 hum 2 10 µM 34 203300 Adrenergic a₃ 319720 rat 2 10 µM 3-15 118002 CYP450, ZOF9 319751 hum 2 10 µM 3-15 118002 CYP450, ZOF9 319751 hum 2 10 µM 3-15 118002 CYP450, ZOF9 319751 hum 2 10 µM 3-15 118002 CYP450, ZOF9 319751 hum 2 10 µM 3-15 118002 CYP450, ZOF9 319751 hum 2 10 µM 3-15 118002 CYP450, ZOF9 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergic a₃ 319751 hum 2 10 µM 3-15 118002 Adrenergi	126000	Beta-Lactamase	319809	ba	2	10 µM	-2		172020	Protein Tyrosine Kinase, Fyn	319773	hum	2	10 µM	5	
1800	112020	Carbonic Anhydrase II	319784	hum	2	10 µM	-5		176020	Protein Tyrosine Kinase, LCK	319785	hum	2	10 µM	0	
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18806 CYP450, 1A2	116020	Cyclooxygenase COX-1	319816	hum	2	10 µM	-12		194020	Thromboxane Synthase	319812	hum	2	10 µM	22	
18870 CYP450, 2C19 319749 hum 2 10 μM 36 200720 Adenosine A ₃ 319695 hum 2 10 μM 39 319806 hum 2 10 μM 39 319806 hum 2 10 μM 39 319806 hum 2 10 μM 38 319806 hum 2 10 μM 38 319806 hum 2 10 μM 38 319806 hum 2 10 μM 39 319806 hum 2 319806 hum 3 319806 h	118010	Cyclooxygenase COX-2	319818	hum	2	10 µM	4		200510	Adenosine A ₁	319717	hum	2	10 µM	15	
118080 CYP450, 2C9 319748 hum 2 10 μM 32 203100 Adrenergic αιλ 319719 rat 2 10 μM 7 118080 CYP450, 2A6 319750 hum 2 10 μM 4 203300 Adrenergic αια 319720 rat 2 10 μM 2 124010 HMG-COA Reductase 319808 hum 2 10 μM 12 203620 Adrenergic αια 319720 rat 2 10 μM 1 132000 Leukotriene LTC, Synthase 319810 gp 2 10 μM 12 203620 Adrenergic αια 319609 hum 2 10 μM 4 133000 Leukotriene LTC, Synthase 319810 gp 2 10 μM 12 203620 Adrenergic αια 319600 hum 2 10 μM 4 133000 Leukotriene LTC, Synthase 319810 gp 2 10 μM 12 203620 Adrenergic αια 319600 hum 2 10 μM 4 14000 Nitric Coxide Synthase, inducible (NOS) 32013 mouse 2 10 μM 6 3 20410 Adrenergic αια 319671 hum 2 10 μM 7 144000 Nitric Coxide Synthase, inducible (NOS) 32013 mouse 2 10 μM 6 204110 Adrenergic βι 319714 hum 2 10 μM 6 1442000 Nitric Coxide Synthase, inducible (NOS) 32013 mouse 2 10 μM 6 204110 Adrenergic βι 319716 hum 2 10 μM 6 1442000 Nitric Coxide Synthase, Neuronal (NOS) 32011 rat 2 10 μM 6 204110 Adrenergic βι 319716 hum 2 10 μM 6 150000 Peptidase, Angiotensin Corverting Enzyme 319781 rabbit 2 10 μM 6 204110 Adrenergic βι 319716 hum 2 10 μM 6 150000 Peptidase, Angiotensin Corverting Enzyme 319781 rabbit 2 10 μM 6 204110 Adrenergic βι 319716 hum 2 10 μM 6 150000 Peptidase, Angiotensin Corverting Enzyme 319781 rabbit 2 10 μM 6 204110 Adrenergic βι 319716 hum 2 10 μM 6 150000 Peptidase, Angiotensin Corverting Enzyme 319781 rum 2 10 μM 6 204110 Adrenergic βι 319716 hum 2 10 μM 6 150000 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 6 204110 Adrenergic βι 319716 hum 2 10 μM 6 150000 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 6 204110 Adrenergic βι 319716 hum 2 10 μM 6 150000 Peptidase, EALX (Neutrophi Elisabse 2) 319757 hum 2 10 μM 6 204110 Adrenergic βι 319700 hum 2 2 10 μM 6 204110 Adrenergic βι 319700 hum 2 2 10 μM 6 204110 Adrenergic βι 319700 hum 2 2 10 μM 6 204110 Adrenergic βι 319700 hum 2 2 10 μM 6 204110 Adrenergic βι 319700 hum 2 2 10 μM 6 204110 Adrenergic βι 319700 hum 2 2 10 μM 6 204110 hum 2 2 10 μM 6 2 204110 Adrenergic βι 319700 hum 2 2 10 μM 6 2	118050	CYP450, 1A2	319747	hum	2	10 µM	-2		200610	Adenosine A _{2A}	319668	hum	2	10 µM	0	
118080 CYP450, 2D6 319750 hum 2 10 μM 4 203200 Adrenergic are 319720 rat 2 10 μM 2 118090 CYP450, 3AA 319751 hum 2 10 μM 38 203400 Adrenergic are 319721 hum 2 10 μM 1-1 132000 Leukotriene LTCs synthase 319808 hum 2 10 μM 12 203620 Adrenergic are 319670 hum 2 110 μM 1-1 132000 Leukotriene LTCs synthase 319810 gp 2 10 μM 4 9 203710 Adrenergic are 319670 hum 2 110 μM 4 3 30000 Lipoxygenase 15-LO 319811 rabbit 2 10 μM 3 204010 Adrenergic are 319670 hum 2 110 μM 3 3 30000 Nitric Oxidase MAO-A 319686 hum 2 10 μM 3 204010 Adrenergic B 319714 hum 2 110 μM 4 14000 Nitric Oxidase MAO-A 319686 hum 2 110 μM 5 204110 Adrenergic B 319714 hum 2 110 μM 6 3 3140010 Nitric Oxidase Synthase, Neuronal (inNOS) 32011 rat 2 10 μM 5 204110 Adrenergic B 319716 hum 2 110 μM 6 3 319714 hum	118070	CYP450, 2C19	319749	hum	2	10 µM	36		200720	Adenosine A ₃	319695	hum	2	10 µM	9	
118090 CYP450, 3A4 319751 hum 2 10 μM 38 203400 Adrenergic ατο 319721 hum 2 10 μM 18 124010 HMG-CoA Reductase 319808 hum 2 10 μM 12 203620 Adrenergic αxa 319606 hum 2 10 μM -1 132000 Leukotriene LTC4 Synthase 319810 gp 2 10 μM 12 203620 Adrenergic αxa 319606 hum 2 10 μM -1 138000 Leukotriene LTC4 Synthase 319810 rabbit 2 10 μM 11 203800 Adrenergic αxa 319607 hum 2 10 μM 4 138000 Leukotriene LTC4 Synthase 319810 rabbit 2 10 μM 11 203800 Adrenergic αxa 319607 hum 2 10 μM 4 138000 Leukotriene LTC4 Synthase 319810 rabbit 2 10 μM 11 203800 Adrenergic αxa 319607 hum 2 10 μM 2 10 μM 3 140010 Monoamine Oxidase MAC-A 319665 hum 2 10 μM 3 204010 Adrenergic αxa 319607 hum 2 10 μM 7 144000 Nitric Oxide Synthase, Neutronal (nNOS) 320013 mouse 2 10 μM 6 204110 Adrenergic β; 319716 hum 2 10 μM 4 142000 Nitric Oxide Synthase, Neutronal (nNOS) 320012 rat 2 10 μM 2 204200 Adrenergic β; 319716 hum 2 10 μM 6 163000 Peptidase, Angiotensin Converting Enzyme 319781 rabbit 2 10 μM 8 210120 Adrenergic β; 319716 hum 2 10 μM 16 163000 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 8 210120 Angiotensin AT2 319909 hum 2 10 μM 5 166010 Peptidase, CTSG (Cathepsin G) 319908 hum 2 10 μM 4 212620 Bradykinin B; 319726 hum 2 10 μM 9 141610 Peptidase, Matrix Metalloproteinase-1 (MMP-1) 320014 hum 2 10 μM 9 14600 Phosphodiesterase PDE3 319808 hum 2 10 μM 9 14600 Phosphodiesterase PDE3 319808 hum 2 10 μM 9 14600 Phosphodiesterase PDE3 319808 hum 2 10 μM 9 14600 Phosphodiesterase PDE3 319808 hum 2 10 μM 9 14600 Phosphodiesterase PDE3 319808 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 14600 Phosphodiesterase PDE5 319809 hum 2 10 μM 9 146	118060	CYP450, 2C9	319748	hum	2	10 µM	32		203100	Adrenergic α _{1A}	319719	rat	2	10 µM	7	
124010 HMG-CoA Reductase 319808 hum 2 110 μM 12 203620 Adrenergic αx 319609 hum 2 110 μM -1 133000 Leukotriene LTC ₄ Synthase 319810 gp 2 110 μM -9 203710 Adrenergic αx 319670 hum 2 110 μM 4 138000 Lipoxygenase 15-LO 319811 rabbit 2 10 μM 11 203800 Adrenergic αx 319670 hum 2 110 μM 4 318000 Lipoxygenase 15-LO 319811 rabbit 2 10 μM 11 203800 Adrenergic αx 319670 hum 2 110 μM 3 3 320400 Adrenergic αx 319670 hum 2 110 μM 3 3 320400 Adrenergic αx 319670 hum 2 110 μM 3 3 320400 Adrenergic αx 319670 hum 2 110 μM 3 3 320400 Adrenergic βx 319715 hum 2 110 μM 4 4 34200 Adrenergic βx 319715 hum 2 110 μM 4 4 34200 Adrenergic βx 319715 hum 2 110 μM 4 4 34200 Adrenergic βx 319715 hum 2 110 μM 6 3 342000 Adrenergic βx 319715 hum 2 110 μM 6 3 3	118080	CYP450, 2D6	319750	hum	2	10 µM	4		203200	Adrenergic a18	319720	rat	2	10 µM	2	
132000 Leukotriene LTC ₄ Synthase 319810 gp 2 10 μM -9 203710 Adrenergic αsc 319670 hum 2 10 μM 4 138000 Lipoxygenase 15-LO 319811 rabbit 2 10 μM 11 203800 Adrenergic αsc 319671 hum 2 10 μM 3 140010 Monoamine Oxidase MAO-A 319665 hum 2 10 μM 3 204010 Adrenergic β1 319714 hum 2 10 μM 7 4 14000 Nitric Oxide Synthase, Neuronal (nNOS) 320012 rat 2 10 μM 6 204110 Adrenergic β2 319715 hum 2 10 μM 7 4 14000 Nitric Oxide Synthase, Neuronal (nNOS) 320012 rat 2 10 μM 6 204110 Adrenergic β2 319716 hum 2 10 μM 7 0 10 μM	118090	CYP450, 3A4	319751	hum	2	10 µM	38		203400	Adrenergic a _{1D}	319721	hum	2	10 µM	18	
138000 Lipoxygenase 15-LO	124010	HMG-CoA Reductase	319808	hum	2	10 µM	12		203620	Adrenergic a2A	319669	hum	2	10 µM	-1	
140010 Monoamine Oxidase MAO-A 319665 hum 2 10 μM 3 204010 Adrenergic β1 319714 hum 2 10 μM 7 144000 Nitric Oxide Synthase, Inducible (INOS) 320013 mouse 2 10 μM 6 204110 Adrenergic β2 319715 hum 2 10 μM 0 144000 Nitric Oxide Synthase, Neuronal (INOS) 320012 rat 2 10 μM 2 204200 Adrenergic β3 319716 hum 2 10 μM 0 107300 Peptidase, Angiotensin Converting Enzyme 319781 rabbit 2 10 μM 0 285010 Adrenergic β3 319716 hum 2 10 μM 16 107300 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 0 285010 Adrenergic β3 319616 hum 2 10 μM 16 103000 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 0 211000 Atrial Natriuretic Factor (ANF) 319903 hum 2 10 μM 9 114110 Peptidase, CASP1 (Caspase 1) 319757 hum 2 10 μM 4 212620 Bradykinin B2 319726 hum 2 10 μM 9 114110 Peptidase, Matrix Metalloproteinase-1 (MMP-1) 320014 hum 2 10 μM 3 214510 Calcium Channel L-Type, Benzothiazepine 319727 rat 2 10 μM 29 114910 Phosphodiesterase PDE3 319828 hum 2 10 μM 2 216000 Calcium Channel L-Type, Phenylalkylamine 320000 rat 2 10 μM 73 171120 Protein Serine/Threonine Kinase, MAPK1 319958 hum 2 10 μM 53 21750 Chemokine CCR2B 320000 hum 2 10 μM 20 17 μM	132000	Leukotriene LTC ₄ Synthase	319810	gp	2	10 µM	l -9		203710	Adrenergic a28	319670	hum	2	10 µM	4	
144000 Nitric Oxide Synthase, Inducible (INOS) 320013 mouse 2 10 μM 6 204110 Adrenergic β2 319715 hum 2 10 μM -4 142000 Nitric Oxide Synthase, Neuronal (InNOS) 32012 rat 2 10 μM 2 204200 Adrenergic β3 319716 hum 2 10 μM 0 107300 Peptidase, Angiotensin Converting Enzyme 319781 rabbit 2 10 μM 0 288010 Androgen (Testosterone) AR 319699 rat 2 10 μM 16 163000 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 8 210120 Angiotensin Arz 319909 hum 2 10 μM 2 166010 Peptidase, CTSG (Cathepsin G) 319908 hum 2 10 μM 4 212620 Bradykinin B2 319726 hum 2 10 μM 9 114110 Peptidase, Matrix Metalloproteinase-1 (IMMP-1) 320014 hum 2 10 μM 3 214510 Calcitum Channel L-Type, Benzothiazepine 319727 rat 2 10 μM 29 152000 Phosphodiesterase PDE3 319828 hum 2 10 μM 9 214600 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 4 73 15600 Phosphodiesterase PDE5 319829 hum 2 10 μM 5 3 19500 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 7 3 19500 Phosphodiesterase PDE5 319829 hum 2 10 μM 5 3 19500 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 7 3 19500 Phosphodiesterase PDE5 319829 hum 2 10 μM 5 3 19500 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 7 3 19500 Phosphodiesterase PDE5 319829 hum 2 10 μM 5 3 19500 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 7 3 19500 Celmokine CCR2B 320000 hum 2 10 μM 7 3 10 μM 7 3 19500 Phosphodiesterase PDE5 319829 hum 2 10 μM 5 2 10 μM 5 2 10 μM 5 3 10 μM 5 2 10 μM 5 3 10 μM 5 3 10 μM 5 3 10 μM 5 2 10 μM 5 3 1	138000	Lipoxygenase 15-LO	319811	rabbit	2	10 µM	11		203800	Adrenergic a2c	319671	hum	2	10 µM	3	
142000 Nitric Oxide Synthase, Neuronal (nNOS) 320012 rat 2 10 μM 2 204200 Adrenergic β3 319716 hum 2 10 μM 0 107300 Peptidase, Angiotensin Converting Enzyme 319781 rabbit 2 10 μM 0 285010 Androgen (Testosterone) AR 319699 rat 2 10 μM 16 163000 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 8 210120 Angiotensin AT₂ 319909 hum 2 110 μM 2-5 112510 Peptidase, CTSG (Cathepsin G) 319908 hum 2 10 μM 0 211000 Atrial Natriuretic Factor (ANF) 319903 gp 2 10 μM 2 166010 Peptidase, ELA2 (Neutrophil Elastase 2) 319775 hum 2 10 μM 4 212620 Bradykinin B₂ 319726 hum 2 10 μM 9 114110 Peptidase, Matrix Metalloproteinase-1 (MMP-1) 320014 hum 2 10 μM 4 212620 Bradykinin B₂ 319726 hum 2 10 μM 2 114910 Peptidase, Matrix Metalloproteinase-9 (MMP-9) 320016 hum 2 10 μM 3 214510 Calcitonin 320010 hum 2 110 μM 26 15200 Phosphodiesterase PDE3 319828 hum 2 10 μM 9 214600 Calcitum Channel L-Type, Benzothiazepine 319727 rat 2 10 μM 47 156000 Phosphodiesterase PDE4 319804 hum 2 10 μM 53 21600 Calcitum Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 47 156000 Phosphodiesterase PDE5 319829 hum 2 10 μM 53 217600 Calcitum Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 73 17100 Protein Serine/Threonine Kinase, MAPK14 319950 hum 2 10 μM 63 217720 Chemokine CCR2B 320006 hum 2 10 μM 14 17100 Protein Serine/Threonine Kinase, MAPK14 319950 hum 2 10 μM 14 171000 Protein Serine/Threonine Kinase, MAPK14 319950 hum 2 10 μM 16 217720 Chemokine CCR5 320008 hum 2 10 μM 14 171000 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 16 218000 Chemokine CCR5 320008 hum 2 10 μM 13 171100 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 16	140010	Monoamine Oxidase MAO-A	319665	hum	2	10 µM	3		204010	Adrenergic β ₁	319714	hum	2	10 μM	7	
107300 Peptidase, Angiotensin Converting Enzyme 319781 rabbit 2 10 μM 0 285010 Androgen (Testosterone) AR 319699 rat 2 10 μM 16 163000 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 8 210120 Angiotensin AT2 319909 hum 2 10 μM -5 112510 Peptidase, CTSG (Cathepsin G) 319908 hum 2 10 μM 0 211000 Atrial Natriuretic Factor (ANF) 319903 gp 2 10 μM 2 166010 Peptidase, ELA2 (Neutrophil Elastase 2) 319757 hum 2 10 μM 4 212620 Bradykinin B2 319760 hum 2 10 μM 9 114110 Peptidase, Matrix Metalloproteinase-1 (MMP-1) 320014 hum 2 10 μM 3 214510 Calcitonin 320010 hum 2 30010 hum 2 10 μM 29 152000 Phosphodiesterase PDE3 319828 hum 2 10 μM 9 214600 Calcitum Channel L-Type, Benzothiazepine 319727 rat 2 10 μM 26 154000 Phosphodiesterase PDE4 319804 hum 2 10 μM 2 215000 Calcitum Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 47 156000 Phosphodiesterase PDE5 319829 hum 2 10 μM 5 217030 Cannabinoid CB₁ Carnel Threonine Kinase, MAPK1 319950 hum 2 10 μM 5 3 217560 Chemokine CCR2B 320006 hum 2 10 μM -2 10	144000	Nitric Oxide Synthase, Inducible (iNOS)	320013	mouse	2	10 µM	6		204110	Adrenergic β ₂	319715	hum	2	10 µM	-4	
163000 Peptidase, CASP1 (Caspase 1) 319813 hum 2 10 μM 8 210120 Angiotensin AT2 319909 hum 2 10 μM -5 112510 Peptidase, CTSG (Cathepsin G) 319908 hum 2 10 μM 0 211000 Atrial Natriuretic Factor (ANF) 319903 gp 2 10 μM 2 166010 Peptidase, ELA2 (Neutrophil Elastase 2) 319757 hum 2 10 μM 4 212620 Bradykinin B2 319726 hum 2 10 μM 9 114110 Peptidase, Matrix Metalloproteinase-1 (MMP-1) 320014 hum 2 10 μM -4 213610 Calcitonin 320010 hum 2 10 μM 29 152000 Phosphodiesterase PDE3 319828 hum 2 10 μM 9 214600 Calcitum Channel L-Type, Benzothiazepine 319727 rat 2 10 μM 26 154000 Phosphodiesterase PDE4 319804 hum 2 10 μM 2 2150 μM 2 215000 Calcitum Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 47 156000 Phosphodiesterase PDE5 319829 hum 2 10 μM -5 217500 Cannabinoid CB1 319676 hum 2 10 μM 73 171120 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 63 21750 Chemokine CCR2B 320006 hum 2 10 μM -1 (CRK) 176610 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, MAPK3 319950 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, MAPK3 319950 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, MAPK3 319950 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, MAPK3 319950 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, MAPK3 319950 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, MAPK3 319950 hum 2 10 μM 71 17100 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 73 17100 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 73 17100 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 73 17100 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 73 17100 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 73 17100 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 74 17100 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 74 17100 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10	142000	Nitric Oxide Synthase, Neuronal (nNOS)	320012	rat	2	10 µM	2		204200	Adrenergic β ₃	319716	hum	2	10 µM	0	
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166010 Peptidase, ELA2 (Neutrophil Elastase 2) 319757 hum 2 10 μM 4 212620 Bradykinin B2 319726 hum 2 10 μM 9 114110 Peptidase, Matrix Metalloproteinase-1 (MMP-1) 320014 hum 2 10 μM -4 213610 Calcitonin 320010 hum 2 10 μM -22 114910 Peptidase, Matrix Metalloproteinase-9 (MMP-9) 320016 hum 2 10 μM 29 152000 Phosphodiesterase PDE3 319828 hum 2 10 μM 9 214600 Calcium Channel L-Type, Benzothiazepine 319727 rat 2 10 μM 26 154000 Phosphodiesterase PDE4 319804 hum 2 10 μM 2 215000 Calcium Channel L-Type, Dihydropyridine 319675 rat 2 10 μM 47 156000 Phosphodiesterase PDE5 319829 hum 2 10 μM -5 217000 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 47 156000 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 53 217500 Chemokine CCR2B 320006 hum 2 10 μM -1 (ERK2) 217660 Chemokine CCR4 320007 hum 2 10 μM -1 (17000 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 71 244500 Chemokine CCR5 (CCKA) 319879 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 3 10 μM -3 10 μM -3 10	163000	Peptidase, CASP1 (Caspase 1)	319813	hum	2	10 µM	8		210120	Angiotensin AT ₂	319909	hum	2	10 µM	-5	
114110 Peptidase, Matrix Metalloproteinase-1 (MMP-1) 320014 hum 2 10 μM -4 213610 Calcitonin 320010 hum 2 10 μM -22 10 μM 29 152000 Phosphodiesterase PDE3 319828 hum 2 10 μM 9 214600 Calcium Channel L-Type, Benzothiazepine 319727 rat 2 10 μM 26 154000 Phosphodiesterase PDE4 319804 hum 2 10 μM 2 215000 Calcium Channel L-Type, Dihydropyridine 319675 rat 2 10 μM 47 156000 Phosphodiesterase PDE5 319829 hum 2 10 μM -5 217000 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 47 171120 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 53 217550 Chemokine CCR2B 320006 hum 2 10 μM -1 (ERK2) 217000 Chemokine CCR2B 320007 hum 2 10 μM -20 176610 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 71 244500 Chemokine CCR5 320008 hum 2 10 μM -1 (ERK1) 218020 Cholecystokinin CCK1 (CCKA) 319772 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, MAPK3 319967 hum 2 10 μM 14 218020 Cholecystokinin CCK1 (CCKA) 319772 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 10 μM -3 10010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 10 μM -3 10010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 10 μM -3 10010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 10 μM -3 10010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 10 μM -3 10010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 10 μM -3 10010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM -3 10 μ	112510	Peptidase, CTSG (Cathepsin G)	319908	hum	2	10 µM	0		211000	Atrial Natriuretic Factor (ANF)	319903	gp	2	10 µM	2	
114910 Peptidase, Matrix Metalloproteinase-9 (MMP-9) 320016 hum 2 10 μM 3 214510 Calcium Channel L-Type, Benzothiazepine 319727 rat 2 10 μM 29 152000 Phosphodiesterase PDE3 319828 hum 2 10 μM 9 214600 Calcium Channel L-Type, Dihydropyridine 319675 rat 2 10 μM 26 154000 Phosphodiesterase PDE4 319804 hum 2 10 μM 2 215000 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 47 156000 Phosphodiesterase PDE5 319829 hum 2 10 μM -5 217030 Cannabinoid CB1 319676 hum 2 10 μM 73 171120 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 53 217550 Chemokine CCR2B 320006 hum 2 10 μM -1 (ERK2) 1776610 Protein Serine/Threonine Kinase, MAPK14 319958 hum 2 10 μM 63 217720 Chemokine CCR4 32007 hum 2 10 μM -1 (ERK1) 10 μM -1 (ERK1) 10 μM 14 14 180010 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 71 218020 Cholecystokinin CCK1 (CCKa) 319677 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 63 219500 Dopamine D1 319677 hum 2 10 μM 6 6	166010	Peptidase, ELA2 (Neutrophil Elastase 2)	319757	hum	2	10 µM	4		212620	Bradykinin B ₂	319726	hum	2	10 µM	9	
152000 Phosphodiesterase PDE3 319828 hum 2 10 μM 9 214600 Calcium Channel L-Type, Dihydropyridine 319675 rat 2 10 μM 26 154000 Phosphodiesterase PDE4 319804 hum 2 10 μM 2 215000 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 47 156000 Phosphodiesterase PDE5 319829 hum 2 10 μM -5 217030 Cannabinoid CB1 319676 hum 2 10 μM 73 171120 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 53 217550 Chemokine CCR2B 320006 hum 2 10 μM -1 (ERK2) 176610 Protein Serine/Threonine Kinase, MAPK14 319958 hum 2 10 μM 63 217720 Chemokine CCR4 320007 hum 2 10 μM -1 (ERK1) 10 μM -1 (ERK1) 10 μM 71 244500 Chemokine CXCR2 (IL-8R _B) 319879 hum 2 10 μΜ 14 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μΜ 63 218020 Cholecystokinin CCK1 (CCKA) 319677 hum 2 10 μΜ 63 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μΜ 63 218020 Cholecystokinin CCK1 (CCKA) 319677 hum 2 10 μΜ 63 2180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μΜ 63 218000 Dopamine D1 319677 hum 2 10 μΜ 63	114110	Peptidase, Matrix Metalloproteinase-1 (MMP-	1) 320014	hum	2	10 µM	-4		213610	Calcitonin	320010	hum	2	10 µM	-22	
154000 Phosphodiesterase PDE4 319804 hum 2 10 μM 2 216000 Calcium Channel L-Type, Phenylalkylamine 320009 rat 2 10 μM 47 156000 Phosphodiesterase PDE5 319829 hum 2 10 μM -5 217030 Cannabinoid CB₁ 319676 hum 2 10 μM 73 171120 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 53 217550 Chemokine CCR2B 320006 hum 2 10 μM -1 (ERK2) 217660 Chemokine CCR4 320007 hum 2 10 μM -20 176610 Protein Serine/Threonine Kinase, MAPK14 319958 hum 2 10 μM 63 217720 Chemokine CCR5 320008 hum 2 10 μM -1 (ERK1) 10 μM 71 244500 Chemokine CXCR2 (IL-8R _B) 319879 hum 2 10 μΜ 14 14 218020 Cholecystokinin CCK1 (CCKA) 319677 hum 2 10 μΜ -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μΜ 63 219500 Dopamine D₁ 319677 hum 2 10 μΜ 6	114910	Peptidase, Matrix Metalloproteinase-9 (MMP-9	9) 320016	hum	2	10 µM	3		214510	Calcium Channel L-Type, Benzothiazepine	319727	rat	2	10 µM	29	
156000 Phosphodiesterase PDE5 319829 hum 2 10 μM -5 217030 Cannabinoid CB₁ 319676 hum 2 10 μM 73 171120 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 53 217550 Chemokine CCR2B 320006 hum 2 10 μM -1 (ERK2) 176610 Protein Serine/Threonine Kinase, MAPK14 319958 hum 2 10 μM 63 217720 Chemokine CCR4 320007 hum 2 10 μM -1 (PARK1) 171000 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 71 244500 Chemokine CCR2 (IL-8R _B) 319879 hum 2 10 μM 14 (ERK1) 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 63 219500 Dopamine D₁ 319677 hum 2 10 μM 6	152000	Phosphodiesterase PDE3	319828	hum	2	10 µM	9		214600	Calcium Channel L-Type, Dihydropyridine	319675	rat	2	10 µM	26	
171120 Protein Serine/Threonine Kinase, MAPK1 319950 hum 2 10 μM 53 217550 Chemokine CCR2B 320006 hum 2 10 μM -1 217660 Chemokine CCR4 320007 hum 2 10 μM -20 217660 Chemokine CCR4 320007 hum 2 10 μM -20 217720 Chemokine CCR5 320008 hum 2 10 μΜ -1 217720 Chemokine CCR5 320008 hum 2 10 μΜ -1 217720 Chemokine CCR5 320008 hum 2 10 μΜ -1 217720 Chemokine CCR5 320008 hum 2 10 μΜ -1 217720 Chemokine CCR5 320008 hum 2 10 μΜ -1 217720 Chemokine CCR5 320008 hum 2 10 μΜ -1 217720 Chemokine CCR2 (IL-8R _B) 319879 hum 2 10 μΜ 14 218020 Cholecystokinin CCK1 (CCKA) 319772 hum 2 10 μΜ -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μΜ 6	154000	Phosphodiesterase PDE4	319804	hum	2	10 µM	2		215000	Calcium Channel L-Type, Phenylalkylamine	320009	rat	2	10 µM	47	
Computing Serine/Threonine Kinase, MAPK3 319958 hum 2 10 μM 21 10 μM 71 218020 Chemokine CCR4 320007 hum 2 10 μM -20 217720 Chemokine CCR5 320008 hum 2 10 μM -10 218020 Chemokine CCR5 320008 hum 2 10 μM -10 218020 Chemokine CCR5 320008 hum 2 10 μM -10 218020 Chemokine CCR2 (IL-8R _B) 319879 hum 2 10 μM -3 218020 Chemokine CCR4 320007 hum 2 10 μM -10 218020 Chemokine CCR2 (IL-8R _B) 319879 hum 2 10 μM -3 318071 hum 2 10 μM -3 318071 hum 2 31949 hum 2 310 μM -3 318071 hum 2 310 μM 6 318071 hum 319071 hum 2 310 μM 6 318071 hum 319071 hum 3190	156000	Phosphodiesterase PDE5	319829	hum	2	10 µM	-5		217030	Cannabinoid CB ₁	319676	hum	2	10 µM	73	
176610 Protein Serine/Threonine Kinase, MAPK14 319958 hum 2 10 μM 63 217720 Chemokine CCR5 320007 hum 2 10 μM -1 (2 RRt) 171000 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 71 244500 Chemokine CCR5 (11-8Rt) 319879 hum 2 10 μΜ 14 (2 RRt) 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 16 219500 Dopamine D1 319677 hum 2 10 μΜ 6	171120	Protein Serine/Threonine Kinase, MAPK1	319950	hum	2	10 µM	53		217550	Chemokine CCR2B	320006	hum	2	10 µM	-1	
(p38α) 171000 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 71 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 16 217720 Chemokine CCK3 320008 hum 2 10 μM 14 244500 Chemokine CXCR2 (IL-8R _B) 319879 hum 2 10 μΜ 14 218020 Cholecystokinin CCK1 (CCKA) 319772 hum 2 10 μΜ -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μΜ 6									217660	Chemokine CCR4	320007	hum	2	10 µM	-20	
171000 Protein Serine/Threonine Kinase, MAPK3 319949 hum 2 10 μM 71 244500 Chemokine CXCR2 (IL-8R _B) 319879 hum 2 10 μM 14 218020 Cholecystokinin CCK1 (CCKa) 319772 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 6	176610		319958	hum	2	10 µM	63		217720	Chemokine CCR5	320008	hum	2	10 µM	-1	
(ERK1) 218020 Cholecystokinin CCK1 (CCKa) 319772 hum 2 10 μM -3 180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 16 219500 Dopamine D1 319677 hum 2 10 μM 6	474000		240040	hum	2	4014	74		244500	Chemokine CXCR2 (IL-8R _B)	319879	hum	2	10 µM	14	
180010 Protein Serine/Threonine Kinase, PRKCA 319967 hum 2 10 μM 16 219500 Dopamine D1 319677 hum 2 10 μM 6	1/1000	r rotour common randos, ma a rec	319949	num	2	10 µM	/1		218020	Cholecystokinin CCK ₁ (CCK _A)	319772	hum	2	10 μM	-3	
(DICC.)	180010	, ,	319967	hum	2	10 µM	16		219500	Dopamine D ₁	319677	hum	2	10 μM	6	
(PKCd) 219600 Dopamine D _{2L} 319854 hum 2 10 μM 4		(PKCa)			_				219600	Dopamine D ₂ L	319854	hum	2	10 μM	4	



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 ETA	319792	hum	2	10 µM	16	
226010	Estrogen ERa	319733	hum	2	10 µM	21	
226810	GABAA, Chloride Channel, TBOB	319722	rat	2	10 µM	34	
226600	GABAA, Flunitrazepam, Central	319681	rat	2	10 µM	-1	
226500	GABAA, 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 I2, 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 MC₅	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 Ms	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 ō₁ (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 ₂ x	319697	rabbit	2	10 µM	-17	
271110	Serotonin (5-Hydroxytryptamine) 5-HT1A	319807	hum	2	10 µM	0	
271200	Serotonin (5-Hydroxytryptamine) 5-HT ₁₈	319835	rat	2	10 µM	-24	
271700	Serotonin (5-Hydroxytryptamine) 5-HT28	319692	hum	2	10 µM	4	
271800	Serotonin (5-Hydroxytryptamine) 5-HT ₂ c	319730	hum	2	10 µM	9	
271910	Serotonin (5-Hydroxytryptamine) 5-HT ₃	319778	hum	2	10 µM	19	
272000	Serotonin (5-Hydroxytryptamine) 5-HT ₄	319825	gp	2	10 µM	22	
272200	Serotonin (5-Hydroxytryptamine) 5-HT ₆	319911	hum	2	10 µM	14	
278110	Sigma σ_1	319711	hum	2	10 µM	15	
278200	Sigma σ 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	

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

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.