Supplementary Figures

Supplementary Figure 1

A

EC50 (nM) = 4.26 ±3.40
Goserelin
HEK293-GnRHR

B

EC50 (nM) = 0.74 ±0.44
Kisspeptin-10
HEK293-KISS1R

C

General Statistic

Agonist mode	GnRHR screening	KISS1R screening
CV (DMSO)	13.33 ± 2.91	31.23 ± 14.87
Z'	0.74 ± 0.051	0.73 ± 0.098
S/B or B/I	31.53 ± 3.70	26.77 ± 19.62
N	408	408

D

Reproducibility

Agonist mode	GnRHR screening	KISS1R screening
Active match	1.51%	0.51%
Inactive match	96.22%	98.22%
Mismatch	0.00%	0.00%
Inconclusive	2.27%	1.27%
AC50 fold difference	1.56	1.32

Supplementary Figure 2

A

Gonadorelin

% Ca²⁺ assay ratio vs Conpound conc. (M)

Fertirelin

% Ca²⁺ assay ratio vs Conpound conc. (M)

Legend: KISS1R, WT, GnRHR

B

Acetylcholine

Legend: KISS1R, WT, GnRHR

Methacholine

Carbachol

Bethanechol

(2-(Acryloyloxy)ethyl)trimethylammonium

Supplementary Figure 3

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┌─────────────────────────┐
│      Tox21 10 K         │
│   compound library      │
└─────────────────────────┘
             │
             ▼
┌──────────────────────────────────────────┐
│  curve rank (target) > 3.5      AND       │
│  Efficacy > 40%                           │
│                 AND                        │
│  curve rank (WT) < 0.5          OR        │
│  efficacy (WT) < 40%            OR        │
│  EC₅₀(WT)/EC₅₀(target) > 1.7              │
└──────────────────────────────────────────┘
             │
             │   ┌──────────────────────────┐
             │   │  Unique & available in   │
             │   │  our compound manager    │
             │   └──────────────────────────┘
      ┌──────┴──────┐
      ▼             ▼
┌────────────┐  ┌────────────┐
│ 55 GnRHR   │  │ 11 KISS1R  │
│  agonists  │  │  agonists  │
└────────────┘  └────────────┘
```

curve rank (target) > 3.5   AND
Efficacy > 40%

AND

curve rank (WT) < 0.5   OR
efficacy (WT) < 40%   OR
$EC_{50}(WT)/EC_{50}(target) > 1.7$

Unique & available in our compound manager

55 GnRHR agonists

11 KISS1R agonists

Supplementary Figure 4

A

Gonadorelin

p-ERK assay: EC50=30.04 ± 17.91 nM

Fertirelin

p-ERK assay: EC50=7.49 ± 7.11 nM

B

1-Dodecyl-3-methylimidazolium

p-ERK assay: EC50=70.19 ± 17.53 µM

1-Methyl-3-tetradecylimidazolium

p-ERK assay: EC50=62.55 ± 35.44 µM

1-Hexadecyl-3-methylimidazolium

p-ERK assay: EC50=50.25 ± 20.77 µM

Cetylpyridinium

p-ERK assay: EC50=59.23 ± 27.83 µM

Myristyltrimethylammonium

p-ERK assay: EC50=52.83 ± 3.51 µM

Hexadecyltrimethylammonium

p-ERK assay: inactive

Ethylhexadecyldimethylammonium

p-ERK assay: EC50=31.36 ± 8.33 µM

Methyltrioctylammonium

p-ERK assay: EC50=30.03 ± 18.15 µM

Supplementary Figure 5

A

Mercuric iodide

B

1-Hexadecyl-3-methylimidazolium

C

Ethylhexadecyldimethylammonium

D

Hexadecyltrimethylammonium

E

Myristyltrimethylammonium

Supplementary Figure 6

A *Gnrh1* expression

- ● DMSO
- ■ 10 nM Kisspeptin-10
- ◆ 50 µM Musk ambrette
- ◆ 25 µM Musk ambrette
- ◆ 12.5 µM Musk ambrette
- ◆ 6.25 µM Musk ambrette

B *Gnrh1* expression

EC_{50} = 21.94 µM

C *GNRH1* expression

p=0.0497
p=0.0324

D

Supplementary Figure 7

A

DMSO

0.1 µg/ml Musk Ambrette

0.3 µg/ml Musk Ambrette

0.5 µg/ml Musk Ambrette

1 µg/ml Musk Ambrette

B

	DMSO	0.1 µg/ml Musk Ambrette	0.3 µg/ml Musk Ambrette	0.5 µg/ml Musk Ambrette	1 µg/ml Musk Ambrette
Swim bladder (%)	100	99	85	72	23
No Swim bladder (%)	NA	1	15	28	77

Supplementary Figure 8