Supporting Information

Discovery of 6-([4-[2-(tert-butylphenyl]-1H-benzimidazol-4-yl)piperazin-1-yl]methyl)quinoxaline (WAY-207024): An orally active antagonist of the Gonadotropin Releasing Hormone Receptor (GnRH-R)

Jeffrey C. Pelletier*, Murty V. Chengalvala*, Joshua E. Cottom*, Irene B. Feingold#,
Daniel M. Green†, Diane B. Hauze*, Christine A. Huseiton*, James W. Jetter‡,
Gregory S. Kopf*, Joseph T. Lundquist IV*, Ronald L. Magolda*, Charles W. Mann†,
Wrobelt

Contents

1. HPLC purity list for compounds 2-9 (Page S2)

2. Experimental details (page S3)

3. Analytical trace and spectra for compound 6 (page S13)
HPLC Purity List

Method A: Column: Xterra MS C18, 5μ, 50 x 2.1 mm. Mobile phase: 90/10-5/95 water (0.1% formic acid)/acetonitrile (0.1% formic acid), 2 minutes, hold 1.5 minutes. Flow rate: 0.8 mL/min. Detection: 210-400 nm.

Method B: Column Xterra reverse phase C18, 3.5μ, 150 x 2.1 mm. Mobile phase: 78/22 - 5/95 phosphate buffer (pH = 2.1) acetonitrile and methanol (1:1) for 10 minutes, hold 4 minutes, 1.2 mL/minute. Detection: 210-370 nm.

<table>
<thead>
<tr>
<th>Compound #</th>
<th>Structure</th>
<th>% purity by HPLC</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td><img src="image" alt="Structure 2" /></td>
<td>100</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Structure 3" /></td>
<td>100</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Structure 4" /></td>
<td>100</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Structure 5" /></td>
<td>100</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Structure 6" /></td>
<td>100</td>
<td>B</td>
</tr>
</tbody>
</table>
Experimental details

General Methods:

All experiments were conducted in well ventilated fume hoods. Anhydrous solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used directly. Bulk solvents and chemicals were purchased from EMD and used directly. $^1$H and $^{13}$C NMR were recorded on Varian INOVA 400 MHz, Bruker AVANCE II 400 MHz and Bruker AVANCE II 300 MHz instruments in the indicated solvent at 20 °C. Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane (TMS). High resolution mass spec were recorded on an Agilent 6210 TOF instrument. Positive and negative electrospray mass spec were recorded on Waters ZQ or ZMD instruments.

The following HPLC and LC/MS methods were used for the syntheses outlined in the Examples:

Method A: Column: Xterra MS C18, 5µ, 50 x 2.1 mm. Mobile phase: 90/10-5/95 water (0.1% formic acid)/acetonitrile (0.1% formic acid), 2 minutes, hold 1.5 minutes. Flow rate: 0.8 mL/min. Detection: 210-400 nm.
Method B: Column Xterra reverse phase C18, 3.5 μ, 150 x 2.1 mm. Mobile phase: 78/22 - 5/95 phosphate buffer (pH = 2.1) acetonitrile and methanol (1:1) for 10 minutes, hold 4 minutes, 1.2 mL/minute. Detection: 210-370 nm.

Method C: Column: YMC CombiPrep ProC18 50 x 20 mm I.D. S-5 μm, 12 nm. Mobile phase: 10/90 acetonitrile/water to 100% acetonitrile over 10 minutes, ramp back to 10/90 acetonitrile/water over 2 minutes. Detection: 210, 254 nm.

Method D: Column: YMC CombiPrep ProC18 50 x 20 mm I.D. S-5 μm, 12 nm. Mobile phase: 10/90 acetonitrile (0.1% TFA)/water (0.1% TFA) to 100% acetonitrile over 10 minutes, ramp back to 10/90 acetonitrile/water over 2 minutes. Detection: 210, 254 nm.

6-((4-[2-(4-tert-butylyphenyl)-1H-benzimidazol-4-yl]piperazin-1-yl)methyl)quinoxaline (6): A solution of 4-((4-(2-(4-tert-butylyphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)benzene-1,2-diamine (13b, 0.40 g, 0.88 mmol) in methanol (5 mL) was treated with 40% aqueous glyoxal (0.21 g, 1.8 mmol, 0.21 mL) and stirred for an hour. The solvent was evaporated and the residue was dissolved in ethyl acetate (50 mL), washed with water (50 mL), dried (MgSO₄) and evaporated. The product was purified by silica gel chromatography eluted with ethyl acetate to leave the purified product 6 as a solid (0.39 g, 93%). ¹H NMR (400 MHz, DMSO-d₆) δ = 12.69 (bs, 1H), 8.95 (d, 1H, J = 1.8 Hz), 8.93 (d, 1H, J = 1.8 Hz), 8.10 (d, 1H, J = 8.6 Hz), 8.06 (s, 1H), 8.04 (d, 2H, J = 8.4 Hz), 7.92 (d, 1H, J = 8.6 Hz), 7.54 (d, 2H, J = 8.5 Hz), 7.05 (m, 1H), 7.03 (d, 1H, J = 5.6 Hz), 6.51 (d, 1H, J = 5.6 Hz), 3.85 (s, 2H), 3.61 (m, 4H), 2.72 (m, 4H), 1.32 (s, 9H). ¹³C NMR (400 MHz, DMSO-d₆) δ = 151.98, 148.05, 145.61, 145.21, 142.69, 142.12, 141.58, 140.90, 136.04, 135.24, 131.32, 128.86, 128.18, 127.50, 125.91, 125.52, 123.06, 106.20, 103.31, 61.61, 52.74, 49.06, 34.43, 30.90. HPLC (Method B) Rᵣ = 9.3 min. (purity = 100.0%).
Methyl 2-(5-((4-(2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-2-nitrophenylamino)acetate (12): To a solution of 2-(4-tert-butylphenyl)-4-(4-(3-fluoro-4-nitrobenzyl)piperazin-1-yl)-1H-benzo[d]imidazole (11, 0.50 g, 1.03 mmol) in N,N-dimethylformamide (20 mL) was added glycine methyl ester hydrochloride (0.515 g, 4.10 mmol). Stirring was begun and to the homogeneous solution was added MP-Carbonate resin (2.01 g, 3.06 mmol/g) and the reaction stirred for three hours. The resin was removed by filtration and the resulting solution heated to sixty degrees and stirred overnight. The solution was diluted with ethyl acetate (100 mL) and washed with water (3 × 30 mL), brine (30 mL), dried (MgSO₄), and evaporated under reduced pressure. The crude material was purified by flash column chromatography using a 20-25% ethyl acetate in dichloromethane gradient to give the pure product 12 as a yellow-orange solid (0.47 g, 82%). ¹H NMR (300 MHz, DMSO-d₆) δ 12.69 (s, 1H), 8.05 (m, 3H), 7.54 (d, 2H, J = 5.6 Hz), 7.03 (m, 2H), 6.91 (m, 1H), 6.78 (dd, 1H, J = 8.8 Hz, J = 1.4 Hz), 6.50 (dd, 1H, J = 7.0 Hz, J = 1.7 Hz), 4.32 (d, 2H, J = 5.8 Hz), 3.72 (s, 3H), 3.58 (m, 6H), 2.64 (m, 4H), 1.33 (s, 9H). LC/MS (Method A) Rₜ = 1.23 min (purity = 100%), [M-H]⁻ = 555.

6-((4-(2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-3,4-dihydroquinoxalin-2(1H)-one (5): A mixture of 2-(5-((4-(2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-2-nitrophenylamino)acetate (12, 0.47 g, 0.84 mmol) and 10% platinum on carbon (10.165 g) in methanol (40 mL) was hydrogenated under one atmosphere of pressure. The catalyst was filtered, washed (methanol, 2 × 50 mL), and the filtrate concentrated under reduced pressure to afford the crude product. Purification by reverse phase HPLC (Method C) yielded 5 as a white solid (0.16 g, 37%). ¹H NMR (300 MHz, DMSO-d₆) δ 12.31 (s, 1H), 10.42 (s, 1H), 9.61 (s, 1H), 8.07 (d, 2H, J = 8.5 Hz), 7.58 (d, 2H, J = 8.5 Hz), 7.15 (m, 2H), 6.80 (m, 3H), 6.64 (m, 1H), 4.44 (br s, 2H), 4.28 (m, 2H), 3.78 (s, 2H), 3.51 (m, 2H), 3.31 (m, 2H), 3.13 (m, 2H), 1.34 (s, 9H). LC/MS (Method A) Rₜ = 0.99 min (purity = 100%), [M+H]^⁺ = 495.
5-((4-(2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-N-methyl-2-nitroaniline: To a solution of 2-(4-tert-butylphenyl)-4-(4-(3-fluoro-4-nitrobenzyl)piperazin-1-yl)-1H-benzo[d]imidazole (11.7 g, 0.20 g, 0.41 mmol) in N,N-dimethylformamide (5 mL) under nitrogen was added methylvamine (2M in THF, 1.03 mL) and the solution was stirred at room temperature for forty-eight hours. The reaction was partitioned between ethyl acetate (150 mL) and water (75 mL). The organic layer was washed with water (3 X 50 mL), brine (75 mL), dried (MgSO4), and evaporated under reduced pressure to yield the product as a yellow-orange solid (0.19 g, 95%). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.69 (s, 1H), 8.21 (m, 1H), 8.04 (m, 3H), 7.55 (d, 2H, \(J = 8.6\) Hz), 7.03 (m, 3H), 6.74 (dd, 1H, \(J = 8.8\) Hz, \(J = 1.5\) Hz), 6.51 (dd, 1H, \(J = 7.1\) Hz, \(J = 1.5\) Hz), 3.60 (m, 6H), 2.99 (d, 3H, \(J = 5.0\) Hz), 2.67 (m, 4H), 1.33 (s, 9H). LC/MS (Method A) \(R_t = 1.24\) min (purity = 100%), [M+H]^+ = 499.

5-((4-(2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-N-1-methylbenzene-1,2-diamine: 5-((4-(2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-N-methyl-2-nitroaniline (0.38 g, 0.76 mmol), prepared as above was hydrogenated over 5% Pt/C (300 mg) at one atmosphere hydrogen pressure in methanol (25 mL) for four hours. The catalyst was filtered, washed (methanol, 2 X 25 mL), and the filtrate evaporated under reduced pressure to give the product as a yellow solid (0.34 g, 95%). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.67 (s, 1H), 8.04 (d, 2H, \(J = 8.6\) Hz), 7.55 (d, 2H, \(J = 8.6\) Hz), 7.02 (m, 2H), 6.48 (m, 2H), 6.39 (m, 2H), 3.54 (br s, 4H), 3.38 (br s, 2H), 2.72 (d, 3H, \(J = 4.4\) Hz), 2.58 (br s, 4H), 1.33 (s, 9H). LC/MS (Method A) \(R_t = 1.24\) min (purity = 94%), [M+H]^+ = 469.

7-((4-(2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-1-methylquinoxaline-2,3(1H,4H)-dione (2): To a solution of 5-((4-(2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-N-1-methylbenzene-1,2-diamine (0.34 g, 0.73 mmol) in tetrahydrofuran (30 mL) under nitrogen was added 1,1'-oxalylidimidazole (0.28 g, 1.45 mmol) and the solution stirred overnight. The reaction was evaporated under reduced pressure to a small volume and then water (30 mL) added. The resulting heterogeneous mixture was stirred at fifty degrees for thirty
minutes and then the precipitate collected by filtration. The crude precipitate was purified by reverse phase HPLC (Method C) to yield 2 as an off-white solid (0.15 g, 40%). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.68 (s, 1H), 12.02 (s, 1H), 8.04 (d, 2H, J = 8.6 Hz), 7.55 (d, 2H, J = 8.6 Hz), 7.33 (s, 1H), 7.18 (m, 2H), 7.03 (m, 2H), 6.49 (dd, 1H, J = 7.1 Hz, J = 1.6 Hz), 3.57 (m, 9H), 2.64 (br s, 4H), 1.33 (s, 9H).

LC/MS (Method A) \(R_t = 1.12\) min (purity = 100%), [M+H]\(^+\) = 523.

5-((4-(4-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-2-nitro-N-(pyridin-3-ylmethyl)aniline: To a solution of 2-(4-tert-butylphenyl)-4-(4-(3-fluoro-4-nitrobenzyl)piperazin-1-yl)-1H-benzo[d]imidazole (11, \(7^a\) 1.0 g, 2.05 mmol) in N,N-dimethylformamide (15 mL) under nitrogen was added 3-(aminomethyl)pyridine (0.84 mL, 8.20 mmol) and the reaction stirred at room temperature for sixteen hours. The reaction was partitioned between ethyl acetate (100 mL) and water (50 mL). The organic layer was washed with water (3 x 50mL), brine (25 mL), dried (MgSO\(_4\)), and evaporated under reduced pressure. The crude material was purified by flash column chromatography using 5% methanol in dichloromethane to give the pure product as a yellow-orange solid (0.66 g, 56%). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.69 (s,1H), 8.75 (t, 1H, J = 6.2 Hz), 8.64 (d, 1H, J = 1.8 Hz), 8.45 (dd, 1H, J = 4.7 Hz, J = 1.5 Hz), 8.06 (m, 3H), 7.77 (m, 1H), 7.55 (d, 2H, J = 8.6 Hz), 7.36 (m, 1H), 7.03 (m, 2H), 6.92 (s, 1H), 6.68 (d, 1H, J = 8.6 Hz), 6.47 (d, 1H, J = 7.2 Hz), 4.71 (d, 2H, J = 6.2 Hz), 3.49 (m, 6H), 2.47 (br s, 4H), 1.33 (s, 9H). LC/MS (Method A) \(R_t = 1.21\) min (purity = 100%), [M+H]\(^+\) = 576.

5-((4-(4-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-N1-(pyridin-3-ylmethyl)benzene-1,2-diamine: 5-((4-(4-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-2-nitro-N-(pyridin-3-ylmethyl)aniline (1.18 g, 2.05 mmol) prepared above, was hydrogenated over 10% Pt/C (400 mg) at one atmosphere hydrogen pressure in methanol (50mL) for four hours. The catalyst was filtered, washed (methanol, 2 x 50 mL), and the filtrate evaporated under reduced pressure to give the product as an off-white solid (1.12 g, 100%). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 12.69 (s, 1H), 8.60 (d, 1H, J = 1.5 Hz), 8.42 (dd, 1H, J = 4.7 Hz, J = 1.2 Hz), 8.06 (d, 2H, J = 8.4 Hz), 7.77 (d, 1H, J = 7.8 Hz), 7.55 (d, 2H, J = 8.4 Hz), 7.33 (m, 1H), 7.02 (m, 2H), 6.53 (d, 1H, J = 8.1 Hz), 6.47 (d,
1H, J = 5.9 Hz), 6.39 (m, 2H), 5.13 (m, 1H), 4.35 (d, 2H, J = 5.5 Hz), 3.44 (br s, 4H), 3.29 (m, 2H), 2.43 (br s, 4H), 1.33 (s, 9H). LC/MS (Method A) R_f = 1.05 min (purity = 100%), [M+H]^+ = 546.

7-((4-((2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-1-(pyridin-3-ylmethyl)quinoxaline-2,3(1H,4H)-dione (3): To a solution of 5-((4-((2-(4-tert-butylphenyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)-N1-(pyridin-3-ylmethyl)benzene-1,2-diamine (0.99 g, 1.81 mmol) in tetrahydrofuran (30 mL) under nitrogen was added 1,1'-oxalylldimidazole (0.69 g, 3.63 mmol) and the solution stirred overnight. The reaction was evaporated under reduced pressure to a small volume and then water (30 mL) added. The resulting heterogeneous mixture was stirred at fifty degrees for thirty minutes and then the precipitate collected by filtration and dried to afford 3 as an off-white solid (0.65 g, 60%). ^1H NMR (300 MHz, DMSO-d_6) δ 12.29 (s, 1H), 9.85 (s, 1H), 8.74 (br s, 1H), 8.52 (d, 1H, J = 4.3 Hz), 8.09 (d, 2H, J = 8.4 Hz), 7.87 (d, 1H, J = 8.0 Hz), 7.58 (d, 2H, J = 8.4 Hz), 7.45 (m, 1H), 7.34 (m, 3H), 7.15 (m, 2H), 6.64 (dd, 1H, J = 6.8 Hz, J = 1.6 Hz), 5.45 (br s, 2H), 4.38 (m, 4H), 3.33 (m, 2H), 3.10 (m, 2H), 3.03 (m, 2H), 1.34 (s, 9H). LC/MS (Method A) R_f = 0.94 min (purity = 100%), [M+H]^+ = 600.

5-((4-((2-(4-tert-butylphenyl)-1H-benzimidazol-4-yl)piperazin-1-yl)methyl)quinoxaline (9): A solution of 2-amino-3-((4-((2-(4-tert-butylphenyl)-1H-benzimidazol-4-yl)piperazin-1-yl)methyl)phenylamine (13a, 0.05 g, 0.11 mmol) and 40% aqueous glyoxal (0.025 mL, 0.22 mmol) were stirred in methanol (5 mL), under nitrogen, at room temperature for 18 hours. The solvent was removed to give an amber colored solid (0.15 g) This material was adsorbed onto silica and purified by column chromatography eluted with a gradient of 0-5% methanol in chloroform to afford the product as a tan solid (20 mg, 38%). ^1H NMR (300 MHz, DMSO) δ = 8.99 (m, 2H), 8.05 (m, 1H), 8.02 (d, 2H, J = 8.1 Hz), 7.98 (m, 2H), 7.55 (d, 2H, J = 8.5 Hz) 7.05 (m, 2H), 6.52 (dd, 1H, J = 6.7 Hz, J = 2.0 Hz), 4.25 (s, 2H), 3.60 (s, 8H), 1.32 (s, 9H). HPLC (method B) R_f = 5.5 mins., (purity = 97.3%). Calculated mass = 476.62, [M - H]^− = 475, [M + H]^+ = 477.
2-Amino-3-nitro-5-vinylpyridine: A mixture of 3-nitro-5-bromopyridin-2-amine (1.9 g, 8.7 mmol), lithium chloride (0.46 g, 11 mmol) and tetrakis(triphenylphosphine)palladium (0.40 g, 0.35 mmol) in toluene 75 mL was stirred, purged with a nitrogen atmosphere, treated with tri-(n-butyl)vinylstannane (3.6 g, 11 mmol, 3.3 mL) and heated to 100 °C for 16 h. The reaction mixture was cooled to room temperature, poured directly onto a silica gel column and eluted with 75% ethyl acetate in hexanes. The product was isolated as a yellow solid (1.1 g, 77%). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta = 8.45\) (d, 1H, \(J = 2.2\) Hz), 8.42 (d, 1H, \(J = 2.2\) Hz), 6.75 (bs, 2H), 6.62 (dd, 1H, \(J = 17.5\) Hz, \(J = 11.0\) Hz), 5.74 (d, 1H, \(J = 17.5\) Hz), 5.32 (d, 1H, \(J = 11.0\) Hz). HPLC (Method B) \(R_t = 6.7\) mins. Calculated mass = 165, [M + H \(^+\)] = 166.

2,3-Diamino-5-vinylpyridine: Prepared according to the method of Cai\(^{12}\). A mixture of 2-amino-3-nitro-5-vinylpyridine (0.30 g, 1.8 mMol) and tin (II) chloride dihydrtate (2.0 g, 9.0 mMol) in 9:1 ethyl acetate-t-butanol was stirred and cautiously treated with sodium borohydride (34 mg, 0.90 mmol). The reaction mixture was heated to 60 °C for 3 h, cooled to room temperature and diluted with ethyl acetate (30 mL) and 1N sodium hydroxide solution (30 mL). After separation of the organic layer it was washed again with 1N sodium hydroxide solution (30 mL), dried (MgSO\(_4\)) and evaporated to leave a tan solid (0.20 g, 82%). \(^1\)H-NMR (CDCl\(_3\)), \(\delta = 7.66\) (d, 1H, \(J = 1.9\) Hz), 7.04 (d, 1H, \(J = 1.9\) Hz), 6.59 (dd, 1H, \(J = 17.6\) Hz, \(J = 11.0\) Hz), 5.55 (d, 1H, \(J = 17.6\) Hz), 5.11 (d, 1H, \(J = 11.0\) Hz), 4.29 (bs, 2H), 3.30 (bs, 2H).

7-Vinylpyrido[3,2-b]pyrazine-2,3(1H,4H)-dione: A mixture of 2,3-diamino-5-vinylpyridine (0.19 g, 1.4 mmol) and oxalyldiimidazole (0.40 g, 2.1 mmol) in tetrahydrofuran (10 mL) was stirred and refluxed under nitrogen for 3 h. After cooling to room temperature the reaction mixture was diluted with water (10 mL), stirred for 1 h and the precipitate was filtered, washed with water and air-dried to leave the product as a solid (0.21 g, 79%) which was collected in two crops. \(^1\)H-NMR (DMSO-d\(_6\)), \(\delta = 12.40\) (bs, 1H), 11.99 (bs, 1H), 8.29 (d, 1H, \(J = 1.8\) Hz), 7.50 (d, 1H, \(J = 1.8\) Hz), 6.77 (dd, 1H, \(J = 17.7\) Hz,
11.0 Hz), 5.80 (d, 1H, J = 17.7 Hz), 5.33 (d, 1H, J = 11.0 Hz). LC/MS (method A), Rt = 0.53 mins (purity > 99.8%), [M + H]+ = 190, [M-H]− = 188.

2,3-Dioxo-1,2,3,4-tetrahydropyrido[3,2-b]pyrazine-7-carbaldehyde (16): A suspension of the vinylpyridopyrazinedione above (0.14 g, 0.74 mmol) in dioxane (6 mL) and water (3 mL) was treated with 2.5% osmium tetroxide solution in tert-butanol (0.38 mL, 37 μMol) and sodium periodate (0.48 g, 2.2 mmol). After stirring for 3 h the reaction mixture was diluted with water (20 mL) and the product was purified by reversed phase HPLC (method C) using nonbuffered mobile phase. The pure product was isolated as a solid (56 mg, 40%). 1H-NMR (DMSO-d6), δ = 12.80 (bs, 1H), 12.20 (bs, 1H), 10.01 (s, 1H), 8.62 (d, 1H, J = 1.8 Hz), 7.78 (d, 1H, J = 1.8 Hz).

7-[(4-[2-(4-tert-Butylphenyl)-1H-benimidazol-4-yl]piperazin-1-yl)methyl]-1,4- dihydropyrido[2,3-b]pyrazine-2,3-dione (4): A solution of 2-(4-tert-butylphenyl)-4-piperazin-1-yl-1H-benimidazole (14, 7a 28 mg, 84 μMol) and 2,3-Dioxo-1,2,3,4-tetrahydropyrido[3,2-b]pyrazine-7-carbaldehyde (16, 20 mg, 0.10 mmol) in N-methylpyrrolidinone (1 mL) was treated with sodium triacetoxyborohydride (25 mg, 0.12 mmol) and stirred for 16 hours. Water (0.25 mL) was added and the mixture was purified directly by reversed phase HPLC (Method C). To leave the product 4 as a foamy solid (10 mg, 23%). 1H NMR (300 MHz, DMSO) δ = 12.68 (bs, 1H), 8.04 (d, 2H, J = 8.7 Hz), 8.03 (m, 1H), 7.55 (d, 2H, J = 8.7 Hz), 7.49 (d, 1H, J = 1.8 Hz), 7.03 (m, 2H), 6.50 (dd, 1H, J = 7.1 Hz, J = 1.8 Hz), 3.58 (s, 2H), 3.56 (m, 4H), 2.62 (m, 4H), 1.32 (s, 9H). HPLC (Method B) Rf = 5.8 min. (purity = 100%). Calculated mass for [M + H]+ = C29H28N7O2 = 510.2612, found = 510.2605.

6-[(4-[2-(4-tert-Butylphenyl)-1H-benimidazol-4-yl]piperazin-1-yl)methyl]quinoline (7): A solution of 2-(4-tert-butylphenyl)-4-piperazin-1-yl-1H-benimidazole (14, 7a 0.10 g, 0.30 mmol) and quinoline-6-carbaldehyde11 (49 mg, 0.31 mmol) in dichloromethane (10 mL) was treated with sodium triacetoxyborohydride (95 mg, 0.45 mmol) and stirred for 18 hours. The solvent was evaporated, the residue was dissolved in ethyl acetate (50 mL), washed with saturated sodium bicarbonate solution (50
mL) and water (50 mL), dried (MgSO₄) and evaporated. The residue was purified by reversed phase HPLC (method D) and the product fractions were freeze-dried to leave 7 as a tris trifluoroacetate salt (70 mg, 30%). ¹H NMR (400 MHz, CD₃OD) δ = 9.04 (dd, 1H, J = 4.5 Hz, J = 1.2 Hz), 8.62 (d, 1H, J = 8.3 Hz), 8.31 (d, 1H, J = 1.4 Hz), 8.24 (d, 1H, J = 8.8 Hz), 8.08 (d, 2H, J = 8.3 Hz), 8.04 (dd, 1H, J = 8.8 Hz, J = 1.8 Hz), 7.75 (d, 1H, J = 8.3 Hz), 7.74 (d, 2H, J = 8.3 Hz), 7.49 (d, 1H, J = 4.5 Hz), 7.48 (s, 1H), 7.17 (dd, 1H, J = 6.3 Hz, J = 2.3 Hz), 4.74 (s, 2H), 3.66 (bs, 4H), 3.55 (bs, 4H), 1.41 (s, 9H). HPLC (Method B) Rᵣ = 6.5 mins. (purity = 99.7%). Calculated mass for [M - H]⁺ = 474, found [M - H]⁺ = 474.

7-((4-[2-(4-tert-Butylphenyl)-1H-benimidazol-4-yl]piperazin-1-yl)methyl)quinoline (8): A solution of 2-(4-tert-butylphenyl)-4-piperazin-1-yl-1H-benimidazole (14, 7a 62 mg, 0.18 mmol) and quinoline-7-carbaldehyde (29 mg, 0.18 mmol) in dichloromethane (6 mL) was treated with sodium triacetoxyborohydride (57 mg, 0.27 mmol) and stirred for 18 hours. The solvent was evaporated, the residue was dissolved in ethyl acetate (50 mL), washed with saturated sodium bicarbonate solution (50 mL) and water (50 mL), dried (MgSO₄) and evaporated. The residue was purified by reversed phase HPLC (method D) and the product fractions were freeze-dried to leave 8 as a tris trifluoroacetate salt (35 mg, 25%). ¹H NMR (400 MHz, CD₃OD) δ = 9.02 (d, 1H, J = 4.4 Hz), 8.57 (d, 1H, J = 8.2 Hz), 8.32 (2, 1H), 8.20 (d, 1H, J = 8.3 Hz), 8.07 (d, 2H, J = 8.0 Hz), 7.86 (d, 1H, J = 8.3 Hz), 7.75 (d, 2H, J = 8.0 Hz), 7.72 (m, 1H), 7.50 (m, 1H), 7.48 (s, 1H), 7.17 (dd, 1H, J = 6.3 Hz, J = 1.4 Hz), 4.77 (s, 2H), 3.67 (bs, 4H), 3.60 (bs, 4H), 1.41 (s, 9H). HPLC (Method B) Rᵣ = 6.6 min. (purity = 94.8%). ESI neg. MS, [M - H]⁺ = 474.

Biological experimental details:

Human and rat in vitro GnRH affinity and efficacy experimental details have been previously reported in ref. 7a and references cited therein.
Experimental details for structure property relationship assays (PAMPA, rLM and solubility) are described in ref. 9.

Experimental details for the in vivo LH suppression assay can be found in ref. 7a.

Pharmacokinetic experimental details:

Cannulated male Sprague-Dawley rats (200-350 g) were administered test compounds as a bolus IV dose at 1 mg/kg or as an oral dose at 20 mg/kg. The dose volumes were 1 mL/kg and 5 mL/kg for IV and oral administration, respectively. Blood samples (0.6 mL) were collected into EDTA tubes at specified timepoints and centrifuged to obtain plasma. The plasma samples were stored at -70°C until analysis. Plasma samples were analyzed by LC/MS/MS following internal standard addition and protein precipitation with acetonitrile. Samples were quantified against standard calibration curves with a limit of detection of 1 ng/mL based on a 100 µL aliquot of plasma.