Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3845–3850
Bioorganic & Medicinal Chemistry Letters

Identification of 2-(4,5,6,7-tetrahydro-1H-pyrrolo[3,2-*c*]pyridin-3-yl)-ethylamine derivatives as novel GnRH receptor antagonists

Mi Chen,[a] Zhiqiang Guo,[a] Marion C. Lanier,[a] Liren Zhao,[a] Stephen F. Betz,[b] Charles Q. Huang,[a] Colin J. Loweth,[a] Neil J. Ashweek,[a] Xin-Jun Liu,[b] R. Scott Struthers,[b] Margaret J. Bradbury,[c] James W. Behan,[c] Jenny Wen,[d] Zhihong O'Brien,[d] John Saunders[a] and Yun-Fei Zhu[a,*]

[a]*Department of Medicinal Chemistry, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[b]*Department of Endocrinology, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[c]*Department of Neurosciences, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[d]*Department of Preclinic Development, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*

Received 27 March 2007; revised 3 May 2007; accepted 4 May 2007
Available online 10 May 2007

Abstract—A novel series of 2-(4,5,6,7-tetrahydro-1H-pyrrolo[3,2-*c*]pyridin-3-yl)-ethylamine derivatives were designed and synthesized as GnRH receptor antagonists. SAR studies led to a series of highly active molecules against both the rat and human receptors. Furthermore, one potent compound, **17j**, demonstrated dose-dependent LH suppression in castrated rats.
© 2007 Elsevier Ltd. All rights reserved.

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) which plays an important role in human reproduction by regulating the levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). GnRH is released from the hypothalamus and acts at GnRH receptor on the pituitary gland to stimulate the biosynthesis and release of LH and FSH. LH released from the pituitary gland is responsible for the regulation of gonadal steroid production in both males and females, while FSH regulates spermatogenesis in males and follicular development in females. Due to its biological mechanism of action, it is believed that several disease states would benefit from the regulation of GnRH function, particularly in antagonizing its activity. These include sex hormone related diseases such as prostate cancers, endometriosis, and uterine fibroids.

In earlier work, our group has disclosed SAR studies over several series (Fig. 1) such as 6-aminomethyl-7-aryl-pyrrolo[1,2-*a*]pyrimid-4-ones[1] (**1**) and 2-aryl-3-aminomethyl-imidazolo[1,2-*a*]pyrimid-5-ones[2] (**2a**–**b**, **3**) and uracils[3] (**4**) as potent non-peptide human gonadotropin-releasing hormone (hGnRH) receptor antagonists. Furthermore, NBI-42902, a uracil-based analog, demonstrated efficacy in both monkey[4] and human subjects.[5] In general, these classes of molecules were found to be highly species-selective. The binding affinities of these molecules are usually >100-fold more potent for the human receptor than for the rat receptor. Thus, the most convenient and cost effective castrated rat model cannot be applied for in vivo efficacy screening to evaluate LH suppression. Interestingly, non-peptide GnRH receptor antagonists from tryptamine class such as **5** were reported to be less species-selective,[6] presumably they bind to a region of the receptor that is more conservative among different species based on mutagenesis studies.[7] To utilize castrated rats as efficient in vivo screening tool, we report here the design, synthesis, and SAR studies of 2-(4,5,6,7-tetrahydro-1H-pyrrolo[3,2-*c*]pyridine-3-yl)-ethylamine derivatives as novel potent GnRH receptor antagonists for both human and rat receptors. Furthermore, LH suppression in castrated rats by one of the compounds is also demonstrated.

Scheme 1 illustrates the 5-step synthesis of the key intermediate **9**. 4-Oxo-piperidine-1-carboxylic acid tert-butyl

Keywords: Non-peptide GnRH receptor antagonists; 12-(4,5,6,7-Tetrahydro-1H-pyrrolo[3,2-*c*]pyridin-3-yl)-ethylamine.
* Corresponding author. Tel.: +1 858 617 7600; fax: +1 858 658 7619; e-mail: fzhu@neurocrine.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.05.009

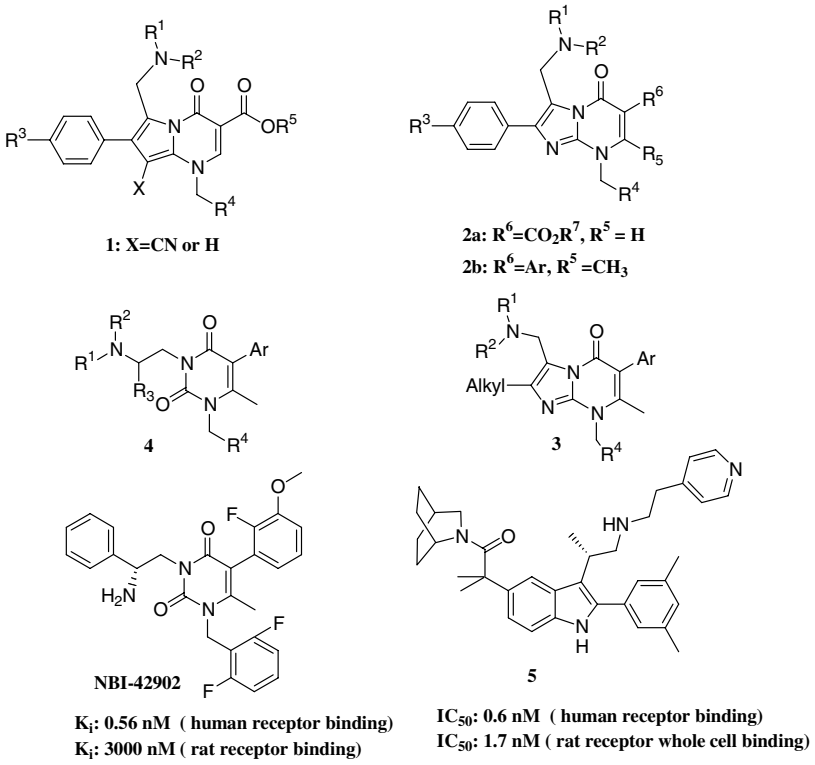
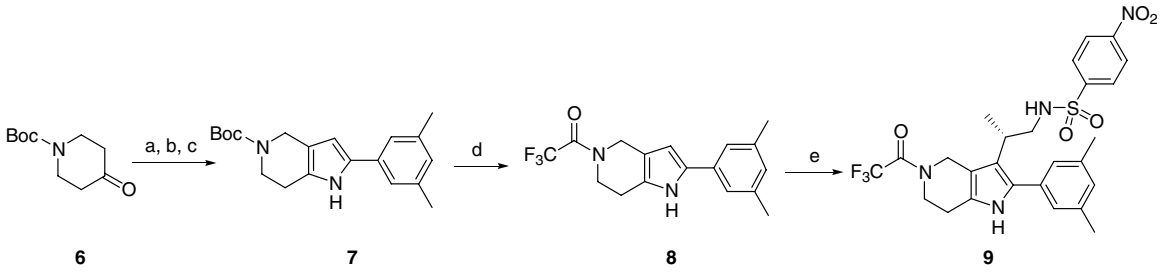
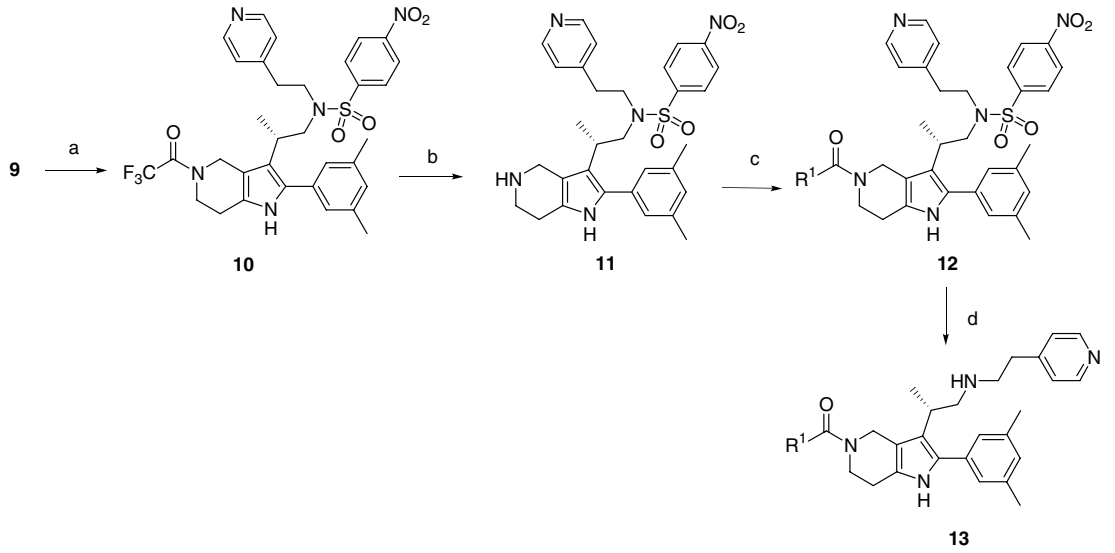


Figure 1. General structures of GnRH antagonists.



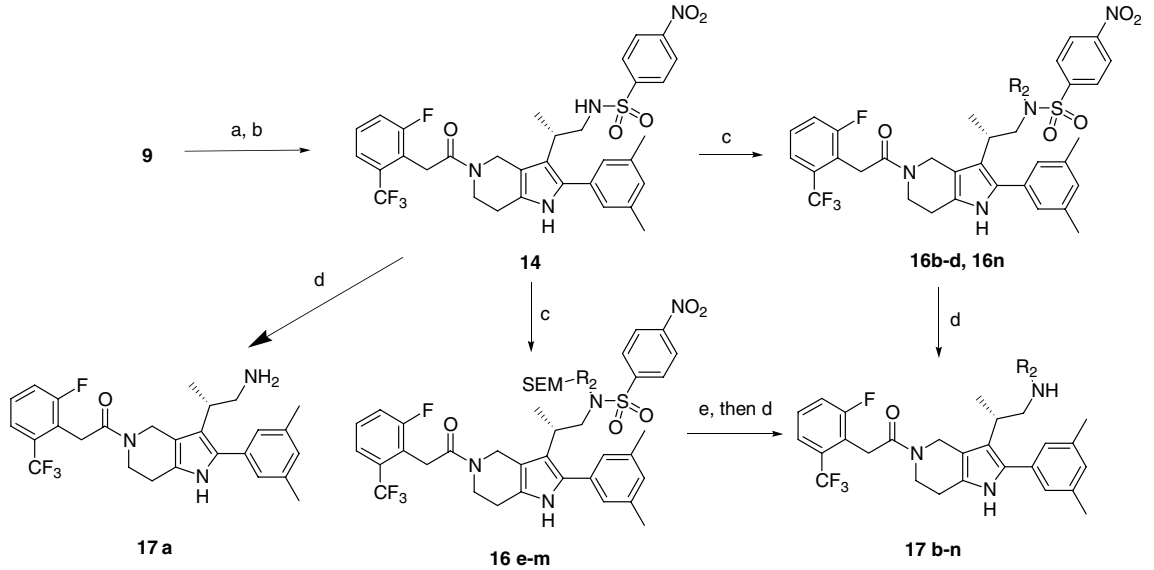
Scheme 1. Reagents and conditions: (a) pyrrolidine, toluene, reflux; (b) 3,5-dimethyl-α-bromoacetophenone, DIPEA, THF, reflux; (c) ammonium acetate, EtOH, 60% from step a to step c; (d) TFA/DCM (1/1), then ethyl trifluoroacetate, MeOH, 78%; (e) $BF_3{\cdot}Et_2O$, (*S*)-1-(4-nitro-benzenesulfonyl)-2-methyl-aziridine, DCM, 75%.

ester (**6**) was heated with pyrrolidine to form the corresponding enamine intermediate, which directly reacted with 3,5-dimethyl α-bromoacetophenone without further manipulation to yield the crude 1,4-diketone product, followed by ring closure with ammonium acetate to afford the pyrrole dervative **7**. N-protecting group of **7** was then switched to acid-stable trifluoroacetamide (**8**), which reacted with the (*S*)-2-methyl-1-(4-nitrobenzenesulfonyl)aziridine[8] to produce the desired compound **9** with orthogonal protections for both basic amines. Two strategies were then adopted for the syntheses of the final compounds **13** and **17** depending on the need for variation of substitutions on a particular basic amino group. Scheme 2 shows the syntheses involving the variation of substitutions on the basic amino group on the bicyclic ring, while the substitution on side chain basic amino group is locked with 2-(4-pyridyl)-ethyl group, one of the preferred groups from tryptamine class.[6a] **9** underwent Mitsunobu reaction with 2-(4-pyridyl)-ethanol to yield **10**. Selective hydrolysis of trifluoroacetamide afforded **11**, which was coupled either with a variety of acids or acyl chlorides to form the corresponding amides. The final compounds **13** were obtained after the hydrolysis of *p*-nitrobenzenesulfonyl group under basic condition in the presence of 2-mercaptoacetic acid. As indicated in Table 1, without substitution on the ring nitrogen, compound **13a** was not active determined by the IP assay which measures the inhibition of GnRH-stimulated [^{3}H] inositol phosphate hydrolysis using RBL cells stably transfected with the gene for human GnRH receptors. Weaker activity was observed with a simple acetamide (**13b**). Subsequently, a variety of amides were examined. Increasing the size of amide from the methyl to cyclopentyl gained about 2-fold activity (**13c**, 3480 nM). A bulky tetramethylcyclopropyl group (**13d**) bolstered the affinity nearly 10-fold to 475 nM (IC_{50}). However, further increase the bulkiness using an adamantyl group did not help on



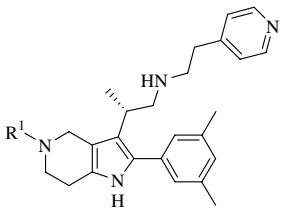
Scheme 2. Reagents: (a) 4-pyridyl-CH_2CH_2OH, Ph_3P, DEAD, THF 30–80%; (b) K_2CO_3, MeOH, 60–80%; (c) amide formation (acyl chloride with Et_3N, DCM or acid with HBTU, DIEA, DCM), 60–95%; (d) LiOH, $HSCH_2CO_2H$, DMF, 50–70%.

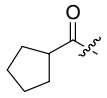
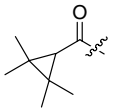
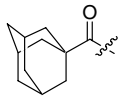
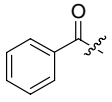
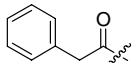
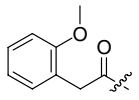
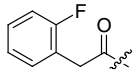
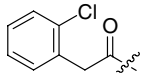
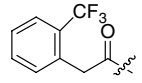
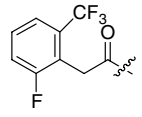
the activity (**13e**, 546 nM). Surprisingly, benzamide (**13f**) was inactive and the activity was regained by an insertion of a methylene group between the phenyl and carbonyl moiety (**10g**, 1110 nM). Based on this lead (**10g**), a variety of substitutions on the phenyl ring were explored. 2-substitution was evidently preferred over 3 and 4-substitutions, thus the discussion in this report is focused on the variation of 2-substitution. Compound **13h** with a 2-methoxyl group improved the affinity by 2-fold, while 2-fluoro (**13i**) and 2-chloro (**13j**) analogs were about 4-fold more potent than the non-substituted one (**13f**). Mutagenesis studies[7] implied that this moiety might be interacting with Tyr^{283}, Tyr^{284}, Phe^{313} residues on the receptor, suggesting that a electron-withdrawing group should be preferred for facilitating a π–π interaction. Indeed, a substantial improvement was observed from the introduction of a 2-CF_3 group (**13k**, IC_{50} = 86 nM). A combination of 2-fluoro and 6-trifluoromethyl groups led to the most potent molecule (**13l**, IC_{50} = 62 nM) from this part of the SAR studies. Subsequently, our attention was turned into optimizing the substitution at the basic amino group on the side chain using the 2-fluoro-6-trifluoromethylphenylacetyl group as the preferred substituent for the central core amino group. Scheme 3 describes the synthetic pathway to obtain such compounds. Thus, **9** was subjected to selective hydrolysis to remove trifluoroacetamide, followed by coupling with 2-fluoro-6-trifluoromethylphenylacetyl chloride to afford **14**. Mitsunobu reactions with selected alcohols (**15b–n**, Table 2) that were obtained either through commercial sources or syntheses[9] produced **16b–n**. For **16b–d** and **16n**, a simple hydrolysis yielded



Scheme 3. Reagents: (a) K_2CO_3, MeOH, 90%; (b) 2-fluoro-6-(trifluoromethyl)phenyl acetic acid, HBTU, DIPEA, DCM, 85%; (c) 4-R^2OH, or SEM-R^2OH, Ph_3P, DEAD, THF 30–80%; (d) LiOH, $HSCH_2CO_2H$, DMF, 50–60%; (e) concd HCl, EtOH, 90%.

Table 1. In vitro activities of **13a–l**



Compound	R^1	hIC_{50}[a] (nM)	rK_i[b] (nM)	rIC_{50}[c,d] (nM)
13a	H	>10,000	>10,000	n.d.
13b		8090	120	n.d.
13c		3480	18	250
13d		475	1.3	96
13e		546	1.5	63
13f		>10,000	660	n.d.
13g		1110	4.5	110
13h		462	5.6	n.d.
13i		271	1.8	280
13j		267	2.6	n.d.
13k		86	1.5	47
13l		62	0.5	57

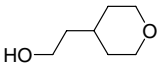
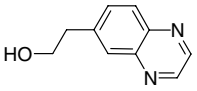
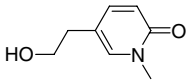
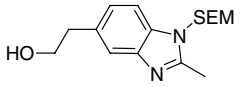
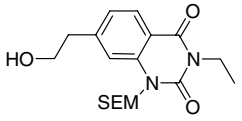
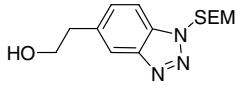
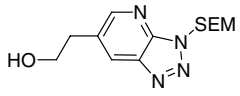
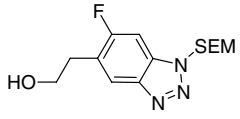
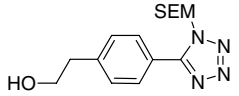
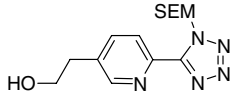
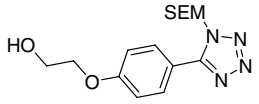
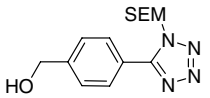
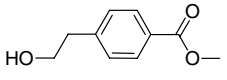
[a] Inhibition of GnRH-stimulated [^{3}H]inositol phosphate hydrolysis.
[b] Inhibition of [^{125}I]Tyr^5,DLeu^6, NMeLeu^7, Pro-*N*-Et-GnRH binding to the cloned rat GnRH receptor.
[c] Inhibition of GnRH stimulated LH release from rat pituitary cells.
[d] n.d., not determined.

the desired products **17b–d** and **17n**. For **16e–m**, which carried a SEM protection group on the heterocycles, an additional step to remove the SEM was employed, followed by the hydrolysis of sulfonamide to yield **17e–m**. Compound **17a** was prepared by a direct hydrolysis of the sulfonamide group on **14**. The inhibitions of GnRH-stimulated [^{3}H] inositol phosphate hydrolysis via human GnRH receptors are summarized in Table 3. While compound **17a** with no N-substitution on the side chain was inactive, attachment of 2-(tetrahydro-pyran-4-yl)-ethyl group, a non-aromatic moiety with a hydrogen bond accepting capability (**17b**), regained some activity, but was less potent than the 2-pyridin-4-yl-ethyl analog (**13l**). While the *N*-methyl pyridinone (**17c**) improved the activity by two-fold, the bicyclic quinoxaline (**17d**) offered no advantage over the pyridyl compound (**13l**). The 2-methylbenzimidazole (**17e**) and 3-ethyl-quinazoline-2,4-dione (**17f**) were similarly potent as **17d** despite their additional hydrogen bond donating capability. However, the benzotriazole analog (**17g**) was more active (IC_{50} = 26 nM) than the benzimidazole (**17e**, IC_{50} = 87 nM), which suggested that an acidic heterocycle might be preferred. Two triazole analogs (**17h–i**) with increased acidity by either inserting a nitrogen or substituting a fluoride on the benzotriazole indeed led to a further improvement of activity. This SAR trend led us to employ a tetrazole moiety which yielded the most active molecule in these SAR studies (**17j**, IC_{50} = 1.5 nM). Further manipulation by insertion of a nitrogen to the phenyl ring (**17k**) decreased the activity (IC_{50} = 10 nM). Attempt to further optimize the chain length between the tetrazole substituted phenyl ring and the basic amino group was not successful, while insertion of an oxygen (**17l**) resulted in a loss of about 40-fold activity and shortening of the chain (**17m**) drastically diminished the activity. Furthermore, replacement of the tetrazole with a carboxylic acid (**17n**) also reduced the activity, indicating the tetrazole group may play additional roles beyond its acidity.

All the compounds were also evaluated by rat binding assay as shown in both Tables 1 and 3. As expected, species selectivity was not an issue for this class of molecules. The rat binding affinities were well correlated with that in human. Rat pituitary cell-based assays measuring the inhibition of GnRH-stimulated LH release were also undertaken before a compound was chosen for in vivo LH suppression study in castrated rats. It was found that this assay offered a better differentiation among the potent binders. For example, both **17d** and **17j** were highly potent rat receptor binders with similar K_i values at 0.3 and 0.2 nM. However, the rat pituitary cell-based assay revealed that **17j** was far more potent than **17d** as demonstrated by their corresponding IC_{50} values (**17j**: 0.6 nM and **17d**: 68 nM). With such small shift of potencies (0.2 nM vs 0.6 nM) from the standard rat receptor binding assay to rat pituitary cell-based functional assay, it was speculated that the binding affinity (K_i) of **17j** was underestimated and should be less than 0.2 nM. However, possibly due to lack of the equilibrium from the standard binding assay condition, its true binding potency was not determined adequately. The similar phenomena were observed in uracil series.[10]

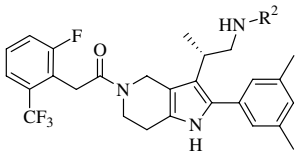
The in vivo efficacy of compound **17j** was assessed by measuring its ability to reduce LH concentrations in rats that had been castrated approximately 4 weeks

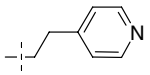
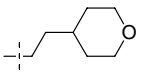
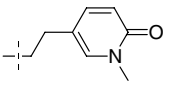
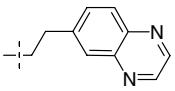
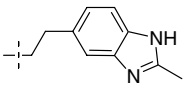
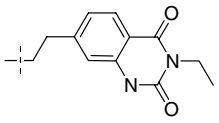
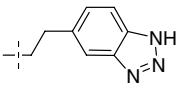
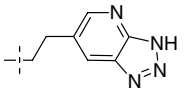
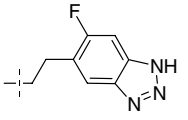
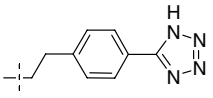
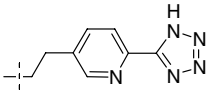
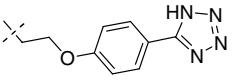
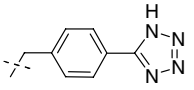
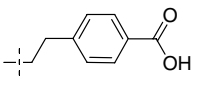
Table 2. The alcohols for Mitsunobu reactions in Scheme 3

Compound	R^2OH
15b	
15c	
15d[6]	
15e[6]	
15f	
15g[6]	
15h	
15i	
15j	
15k	
15l	
15m	
15n	

prior to the study. The castrated rats had elevated LH concentrations that were stable over 24 h. Blood samples from individually housed rats were withdrawn from pre-

Table 3. In vitro activities of **17a–n**



Compound	R^2	hIC_{50}[a] (nM)	rK_i[b] (nM)	rIC_{50}[c] (nM)
13l		62	0.5	57
17a	H	4180	470	n.d.
17b		205	4.7	480
17c		32	0.4	n.d.
17d		60	0.3	68
17e		87	1.0	n.d.
17f		91	1.8	n.d.
17g		26	0.5	1.2
17h		9.0	0.6	6.4
17i		16	0.7	28
17j		1.5	0.2	0.6
17k		7.0	0.6	44
17l		63	6.2	n.d.
17m		1600	1600	n.d.
17n		22	2.0	n.d.

n.d., not determined.

[a] Inhibition of GnRH-stimulated [^{3}H]inositol phosphate hydrolysis.

[b] Inhibition of [^{125}I]Tyr^5,DLeu^6, NMeLeu^7, Pro-*N*-Et-GnRH binding to the cloned rat GnRH receptor.

[c] Inhibition of GnRH stimulated LH release from rat pituitary cells.

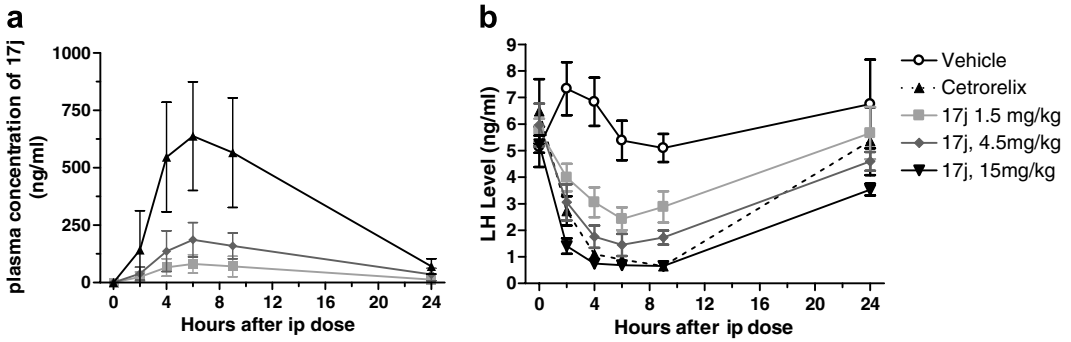


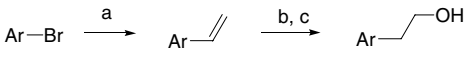
Figure 2. (a) Pharmacokinetics of **17j** in castrated rats via ip administration. (b) LH suppression of **17j** in castrated rats via ip administration.

viously implanted catheters prior to and up to 24 h after intraperitoneal (ip) injection of **17j**, vehicle, or subcutaneous injection of the peptide antagonist Cetrorelix at 10 μg/kg. Compound **17j** dose-dependently decreased LH concentrations as measured by radioimmunoassay with significant effects observed at 1.5 mg/kg, the lowest dose administered ($p < 0.05$, Two-way Repeated Measures ANOVA, Fig. 2b). The highest dose, 15 mg/kg, reduced plasma LH concentrations to the same degree as the positive control Cetrorelix. For all doses, suppression was significant from 2 to 9 h. Plasma concentrations of **17j** after ip injection increased with dose (Fig. 2a). The AUCs ((ng h)/ml) of plasma concentration from rats completing the 24-h study were 1290 ± 221.8 (1.5 mg/kg), 2473.4 ± 373.4 (4.5 mg/kg), and 6502.0 ± 1094.6 (15 mg/kg), respectively.

In summary, we have developed a novel series of GnRH receptor antagonists that were both potent against human and rat receptors in vitro. Effectiveness of such class of molecules to suppress LH was demonstrated in castrated rats.

References and notes

1. (a) Zhu, Y.-F.; Struthers, R. S.; Connors, P. J., Jr.; Gao, Y.; Gross, T. D.; Saunders, J.; Wilcoxen, K.; Reinhart, G. J.; Ling, N.; Chen, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 339; (b) Zhu, Y.-F.; Wilcoxen, K.; Saunders, J.; Guo, Z.; Gao, Y.; Connors, P. J., Jr.; Gross, T. D.; Tucci, F. C.; Struthers, R. S.; Reinhart, G. J.; Xie, Q.; Chen, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 403.
2. (a) Wilcoxen, K.; Zhu, Y.-F.; Connors, P. J., Jr.; Saunders, J.; Gross, T. D.; Gao, Y.; Reinhart, G. J.; Struthers, R. S.; Chen, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2179; (b) Gross, T. D.; Zhu, Y.-F.; Saunders, J.; Gao, Y.; Connors, P. J., Jr.; Guo, Z.; Struthers, R. S.; Reinhart, G. J.; Chen, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2185; (c) Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Gao, Y.; Connors, P. J.; Struthers, R. S.; Xie, Q.; Tucci, F. C.; Reinhart, G. J.; Wu, D.; Saunders, J.; Chen, C. *J. Med. Chem.* **2003**, *46*, 1769.
3. (a) Zhu, Y.-F.; Gross, T. D.; Guo, Z.; Connors, P. J.; Gao, Y.; Tucci, F.; Struthers, R. S.; Reinhart, G. J.; Saunders, J.; Chen, T. K.; Bonneville, A. K.; Chen, C. *J. Med. Chem.* **2003**, *46*, 2023; (b) Guo, Z.; Zhu, Y.-F.; Gross, T. D.; Tucci, F.; Gao, Y.; Moorjani, M.; Connors, P. J.; Rowbottom, M. R.; Chen, Y.; Struthers, R. S.; Xie, Q.; Saunders, J.; Reinhart, G. J.; Chen, T.; Bonneville, A. K.; Chen, C. *J. Med. Chem.* **2004**, *47*, 1259; (c) Rowbottom, M. R.; Tucci, F.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Reinhart, G. J.; Xie, Q.; Struthers, R. S.; Saunders, J.; Chen, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2269; (d) Tucci, F. C.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Connors, P. J., Jr.; Gao, Y.; Rowbottom, M. W.; Struthers, R. S.; Reinhart, G. J.; Xie, Q.; Chen, T. K.; Bozigian, H.; Bonneville, A. L. K.; Fisher, A.; Jin, L.; Saunders, J.; Chen, C. *J. Med. Chem.* **2004**, *47*, 3486.
4. Tucci, F.; Zhu, Y.-F.; Struthers, R. S.; Guo, Z.; Gross, T. D.; Rowbottom, M. R.; Acevedo, O.; Gao, Y.; Saunders, S.; Xie, Q.; Reinhart, G. J.; Liu, X.; Ling, N.; Bonneville, A. K.; Chen, T.; Bozigian, H.; Chen, C. *J. Med. Chem.* **2005**, *48*, 1169.
5. Struthers, R. S.; Chen, T.; Campbell, B.; Jimenez, R.; Pan, P.; Yen, S. S. C.; Bozigian, H. P. *J. Clin. Endocrino. Metabl.* **2006**, *91*, 3903.
6. (a) Ashton, W. T.; Sisco, R. M.; Kieczykowski, G. R.; Yang, Y. T.; Yudkovitz, J. B.; Cui, J.; Mount, G. R.; Ren, R. N.; Wu, T.-J.; Shen, X.; Lyons, K. A.; Mao, A.-H.; Carlin, J. R.; Karanam, B. V.; Vincent, S. H.; Chang, K.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2597, and references therein; (b) Young, J. R.; Huang, S. X.; Walsh, T. F.; Wyvratt, M. J., Jr.; Yang, Y. T.; Yudkovitz, J. B.; Cui, J.; Mount, G. R.; Ren, E. N.; Wu, T.-J.; Shen, X.; Lyons, K. A.; Mao, A.-H.; Carlin, J. R.; Karanam, B. V.; Vincent, S. H.; Cheng, K.; Goulet, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 827.
7. Betz, S.; Reinhart, G. J.; Lio, F. M.; Chen, C.; Struthers, R. S. *J. Med. Chem.* **2006**, *49*, 637.
8. Farr, R. N.; Alabaster, R. J.; Chung, J. Y. L.; Carig, B.; Edwards, J. S.; Gibson, A. W.; Ho, G.-J.; Humphrey, G. R.; Johnson, S. A.; Grabowski, E. J. J. *Tetrahedron Asymmetry* **2003**, *14*, 3503.
9. The general synthesis for alcohols **15c–m** was performed according to the following Scheme[6]:



15c-m

Reagents and conditions: (a) Tributyl(vinyl)tin, $Pd(Ph_3P)_2Cl_2$, DMF; (b) 9-BBN, THF, reflux; (c) H_2O_2, 2 N NaOH, room temperature.
10. Sullivan, S. K.; Hoare, S. R. J.; Fleck, B. A.; Zhu, Y.-F.; Heise, C. E.; Struthers, R. S.; Crowe, P. D. *Biochem. Pharm.* **2006**, *72*, 838.