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A New Class of Potent Nonpeptide Luteinizing Hormone-Releasing Hormone (LHRH) Antagonists: Design and Synthesis of 2-Phenylimidazo[1,2-*a*]pyrimidin-5-ones

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Abstract—The design and synthesis of a new class of nonpeptide luteinizing hormone-releasing hormone (LHRH) receptor antagonists, the 2-phenylimidazo[1,2-*a*]pyrimidin-5-ones, is reported. Among compounds described in this study, we identified the potent antagonist **15b** with nanomolar in vitro functional antagonism. The result might suggest that the heterocyclic 5–6-ring system possessing a pendant phenyl group attached to the five-membered ring is the important structural feature for a scaffold of small molecule LHRH antagonists.

Introduction

In recent years, luteinizing hormone-releasing hormone (LHRH, also known as gonadotropin-releasing hormone; GnRH) antagonists are widely acknowledged as logical candidates for new types of drugs in the treatment of endocrine-based diseases, for example certain sex hormone-dependent cancers, endometriosis, uterine leiomyoma and precocious puberty.[1,2] Indeed, peptidic LHRH antagonists, which are expected to directly reduce the steroid hormone levels without the initial 'flare effect' induced by peptidic LHRH agonists, have achieved clinical success.[2,3] Therefore, it is expected that potent and orally bioavailable nonpeptide LHRH antagonists may be clinically desirable agents without the usual liabilities associated with large peptidic therapeutics.

We previously identified the 2-phenylthieno[2,3-*b*]pyridin-4-one T-98475 (**1**) as the first, potent, and orally active nonpeptide LHRH receptor antagonist.[4] Inspired by this achievement, further effort has been devoted toward searching for new scaffolds of small molecule LHRH antagonists. Recently, we investigated the synthesis and biological activity of a new series of potent nonpeptide LHRH antagonists, the 6-phenylthieno[2,3-*d*]pyrimidine-2,4-diones **2**, which were designed by substituting the pyrimidine ring for the pyridine ring of the thienopyridin-4-one nucleus.[5] Therefore, we next focused on the thiophene ring of the thienopyridin-4-one core and decided to replace it with other five-membered rings. Since the biological activities and pharmacokinetic profiles of compounds depend on their physicochemical properties, incorporation of a five-membered ring with different physicochemical properties from those of the thiophene ring, into a bicyclic scaffold was hoped to provide new potent LHRH antagonists with improved pharmacokinetic profiles. Accordingly, we designed the 2-phenylimidazo[1,2-*a*]pyrimidin-5-one **3**,[6] a heterocyclic 5–6-ring system in which an imidazole ring is embedded, as a novel scaffold for nonpeptide LHRH antagonists.

In this letter, we wish to describe the synthesis and biological evaluation of a series of 2-phenylimidazo[1,2-*a*]pyrimidin-5-ones, which led to the identification of a

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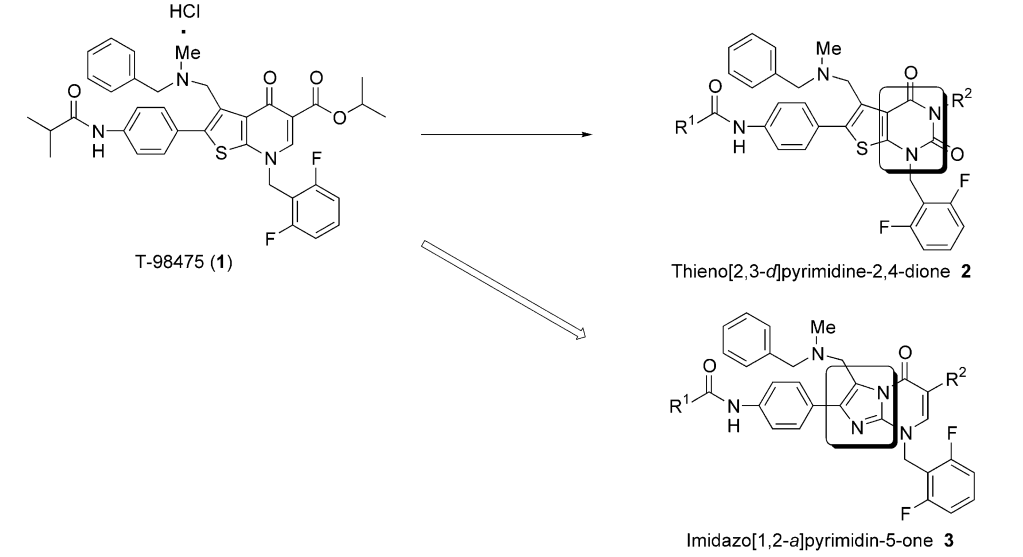
new class of potent low molecular weight LHRH antagonists, exemplified by the methoxyurea **15b**.

Chemistry

To our knowledge, there are no reports on the synthesis of highly functionalized 2-arylimidazo[1,2-*a*]pyrimidin-5-ones.[7] Hence, we started by developing a synthetic procedure to obtain the target compound **IV**, according to the approach illustrated in Chart 1. Briefly, alkylation of 2-amino-4-hydroxypyrimidine **I** with 2-bromopropiophenone **II** provides the 3-alkyl-2-aminopyrimidin-4-one **III**, which can then be converted to the desired imidazopyrimidin-5-one **IV** by intramolecular cyclization.

The methodology of construction of the 2-phenylimidazopyrimidine nucleus is shown in Scheme 1. Initially, reaction of ethyl 2-amino-4-hydroxypyrimidine-5-carboxylate **4** with 2-bromopropiophenone **5** in the presence of potassium carbonate did not produce the desired N^3-alkylated compound and the cyclized



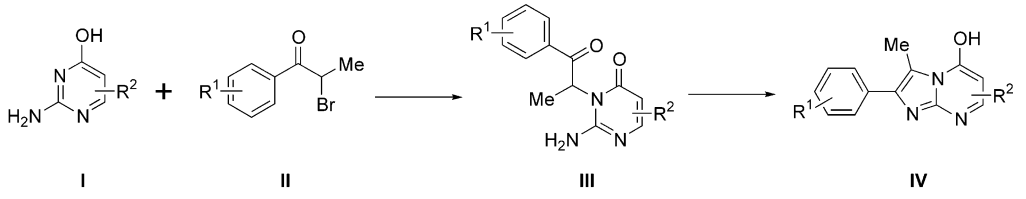
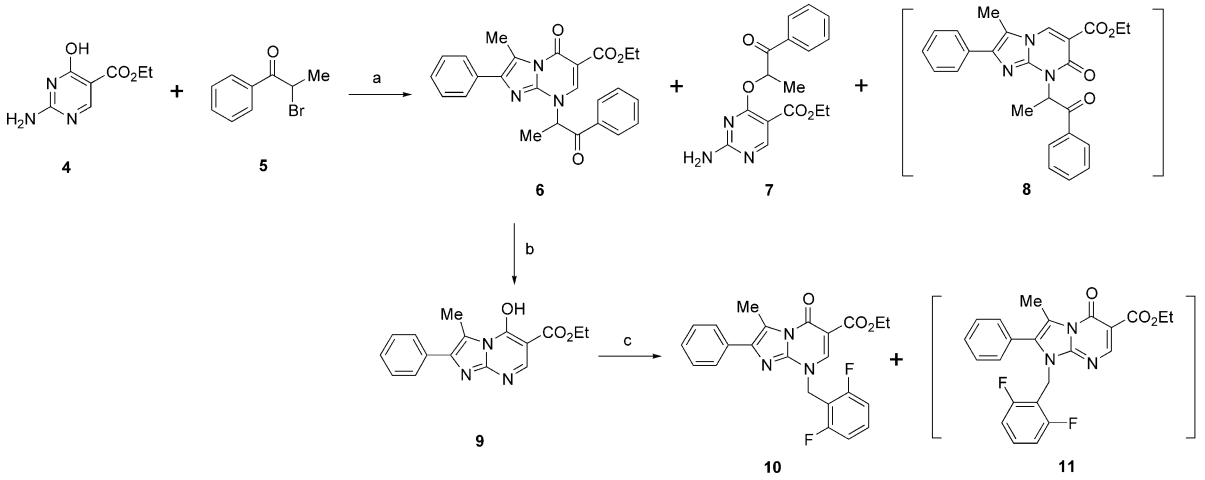


Chart 1.



Scheme 1. Reagents and conditions: (a) K_2CO_3, KI, DMF (**6**: 37%, **7**: 31%, **8**: 1%); (b) Zn, AcOH (78%); (c) 2,6-difluorobenzyl chloride, K_2CO_3, KI, DMF (**10**: 85%, **11**: 8%).

product **9**, but instead gave rise to the imidazopyrimidine **6** and the *O*-alkylated pyrimidine **7** (approximately 1:1 ratio) together with **8** and other minor products.[8] Determination of the chemical structures of **6** and **8** was achieved by measurement of the nuclear Overhauser effect (NOE) between the two methyl groups and the proton on the pyrimidine ring. Although efforts have been made toward preparation of the N^3-alkylated product or **9** directly from **4** and **5** in satisfactory yield, these attempts were unsuccessful. This might be explained as follows: after initial N^3-, *O*-, or N^1-alkylation of **4** occurred (N^3- and *O*-alkylation exceeded N^1-alkylation), ring-closure reaction of the N^3-, N^1-alkylated products and subsequent alkylation with **5** afforded **6** and **8**, respectively. Secondary alkylation may well be attributed to the relatively high reactivity of the imidazopyrimidines due to their electron-rich nature. Based on these results, we next examined conversion of **6** to the desired product **9**. Gratifyingly, reductive dealkylation of the phenacyl group using zinc powder and acetic acid proceeded smoothly to furnish **9** in good yield. Alkylation of compound **9** with 2,6-difluorobenzyl chloride gave the two regioisomers **10** (85%) and **11** (8%).[9] X-ray crystallography showed the major product to be the desired N^8-alkylated compound **10** (see Fig. 1).[10]

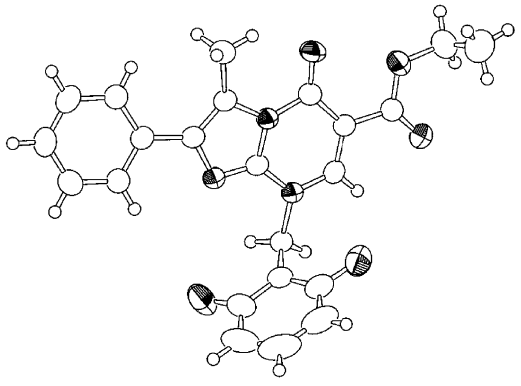
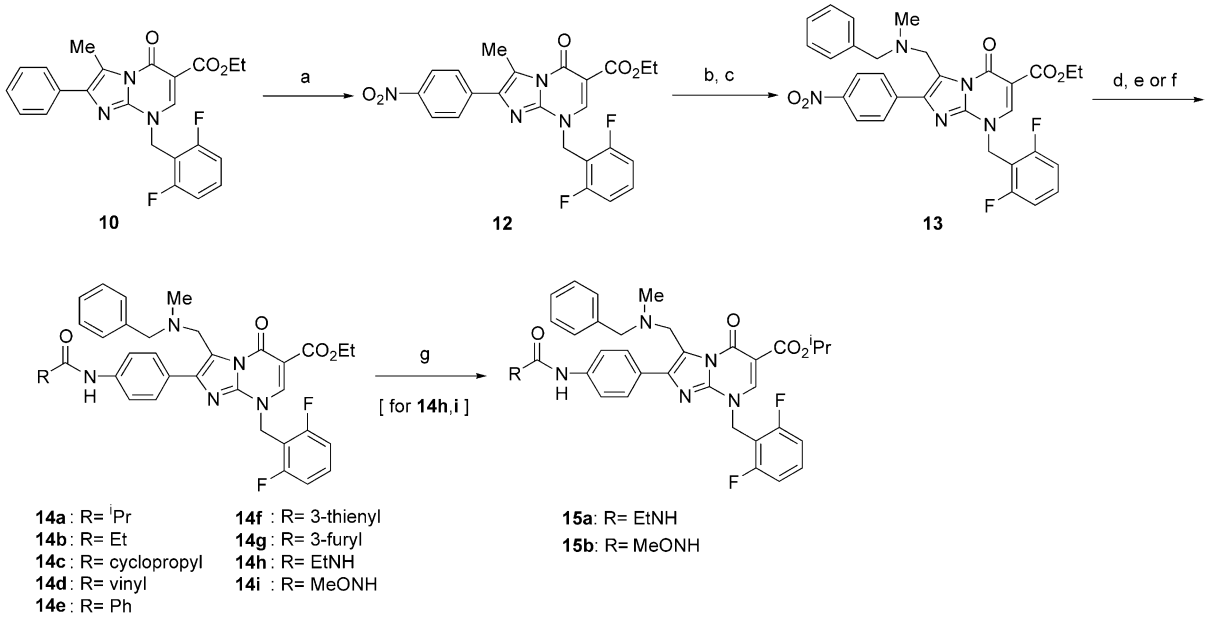


Figure 1. X-ray structure of compound **10**.[10]

The synthetic procedure for the amides **14a–g** and urea analogues **14h,i** and **15a,b** is outlined in Scheme 2. Nitration of **10** with sodium nitrate in sulfuric acid took place with almost complete regioselectivity toward the para position of the 2-phenyl ring to afford **12**. After bromination of **12** and incorporation of the *N*-benzylmethylamino group, the nitro compound **13** was obtained. Reduction of **13** with iron powder-hydrochloric acid provided the corresponding aniline, which in turn was converted to the target amide and urea derivatives **14a–i** via acylation using acyl chlorides, condensation with carboxylic acids, reaction with ethyl isocyanate, or reaction of 1,1′-carbonyldiimidazole (CDI) and successive treatment of *O*-methylhydroxylamine. Finally, transesterification of compounds **14h,i** with titanium(IV) isopropoxide in 2-propanol afforded the isopropyl esters **15a,b**.[11]

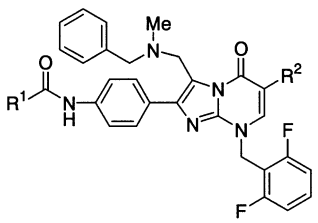
Results and Discussion

The imidazo[1,2-*a*]pyrimidin-5-one derivatives were evaluated for inhibition of specific [^{125}I]leuprorelin binding to the cloned human LHRH receptor[12] and the results are summarized in Table 1. Initially, the para substituent on the 2-phenyl ring of the imidazopyrimidin-5-ones was explored for the 6-ethyl ester derivatives. The isobutyrylamide **14a** and propionylamide **14b** were found to show significant 10^{-8} M order binding



Scheme 2. Reagents and conditions: (a) $NaNO_3$, concd H_2SO_4 (89%); (b) NBS, AIBN, CCl_4; (c) *N*-benzylmethylamine, $^iPr_2NEt$, DMF (80%, two steps from **12**); (d) Fe, concd HCl, EtOH (95%); (e) RCOCl, Et_3N, CH_2Cl_2 (58–61%) or RCO_2H, PyBOP, $^iPr_2NEt$, CH_2Cl_2 (48–86%); (f) EtNCO, Py (65%) or (**1**) CDI, Et_3N, CH_2Cl_2; (2) *O*-methylhydroxylammonium chloride, Et_3N (50%); (g) Ti(O^iPr)$_4$, ^iPrOH (**15a**: 47%, **15b**: 13%).

Table 1. In vitro activities of imidazo[1,2-*a*]pyrimidin-5-one derivatives



Compd	R^1	R^2	Binding affinity IC_{50}[a] (nM)
14a	^{i}Pr	CO_2Et	30
14b	Et	CO_2Et	10
14c	Cyclopropyl	CO_2Et	5
14d	Vinyl	CO_2Et	3
14e	Ph	CO_2Et	20
14f	3-Thienyl	CO_2Et	7
14g	3-Furyl	CO_2Et	3
14h	EtNH	CO_2Et	0.5
14i	MeONH	CO_2Et	0.7
15a	EtNH	$CO_2{}^iPr$	0.3
15b	MeONH	$CO_2{}^iPr$	0.4

[a]The binding affinity is reported as the IC_{50} value, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to the LHRH receptor by 50%. Chinese hamster ovary (CHO) cells expressing human LHRH receptors were used as the source for LHRH receptors. All data are expressed as means of two or three determinations.

affinities. Replacement of the alkyl group of **14a** with cyclopropyl or vinyl moieties (**14c**,**d**) gave 6- to 10-fold enhancement in activity. This indicated that the smaller alkylamides are favorable. In the arylamide series, the benzoyl analogue **14e** was less potent than **14c**,**d**, however, substitution of the phenyl group of **14e** for the thiophene (**14f**) or furan (**14g**) rings increased the affinity. The high binding affinities of **14f**,**g** might be a consequence of the smaller ring size and/or introduction of the hetero atom.

Incorporation of urea and urea-related moieties onto the 2-phenyl ring produced an increase in activity. The ethylurea **14h** and methoxyurea **14i** displayed sub-nanomolar affinities and were 14- to 20-fold more potent than **14b**. Moreover, transesterification of **14 h**,**i** caused further enhancement in affinity. The isopropyl esters **15a**,**b** were about twice as potent as the ethyl esters **14h**,**i**. Binding affinities of **15a**,**b** were almost comparable to that of T-98475 (**1**) (IC_{50} value of 0.2 nM). The result revealed that the imidazopyrimidin-5-one is a new scaffold for nonpeptide LHRH antagonists in addition to the thienopyridin-4-one and thienopyrimidine-2,4-dione scaffolds.

The ethylurea **15a** and methoxyurea **15b**, which exhibited low sub-nanomolar affinities, were next evaluated for in vitro functional antagonism.[13] Compounds **15a**,**b** potently inhibited LHRH-stimulated arachidonic acid release from CHO cells expressing the human LHRH receptor, with IC_{50} values of 10 and 7 nM, respectively. From these data, the imidazopyrimidin-5-ones **15a**,**b** proved to be potent nonpeptide LHRH antagonists in this context.

Consequently, this study has demonstrated that the imidazopyrimidin-5-ones, thienopyridin-4-ones, and thienopyrimidine-2,4-diones constitute a new class of potent nonpeptide LHRH antagonists. It is expected that further optimization of the 6-substituent of **15a**,**b** will lead to identification of potent and orally effective LHRH antagonists.

Conclusion

Starting with the thienopyridine-based LHRH antagonist T-98475 (**1**), a series of the 2-phenylimidazopyrimidin-5-ones was designed, synthesized, and evaluated as nonpeptide LHRH antagonists. This study resulted in the identification of a new class of potent LHRH antagonists, represented by the methoxyurea **15b** possessing high binding affinity and potent in vitro antagonism, with IC_{50} values of 0.4 and 7 nM, respectively. These results led us to conclude that the imidazopyrimidin-5-one provides a new scaffold for small molecule LHRH antagonists devoid of a thiophene ring. Taking this finding into consideration, it is suggested that the heterocyclic 5–6-ring system bearing a pendant phenyl group attached to the five-membered ring is the key structural motif for a LHRH antagonist scaffold.

Acknowledgements

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8. The physicochemical data of compounds **6**, **7** and **8** were as follows. Compound **6**: mp 177–178 °C; ^{1}H NMR (300 MHz; TMS/$CDCl_3$) δ 1.42 (3H, t, J = 7.1 Hz), 1.83 (3H, d, J = 7.6 Hz), 2.90 (3H, s), 4.41 (2H, q, J = 7.0 Hz), 6.82 (1H, q,

$J = 7.3$ Hz), 7.26–7.43 (3H, m), 7.52–7.70 (5H, m), 8.13 (2H, d, $J = 8.8$ Hz), 8.54 (1H, s). Compound **7**: ^{1}H NMR (300 MHz; TMS/$CDCl_3$) δ 1.35 (3H, t, $J = 7.2$ Hz), 1.70 (3H, d, $J = 7.1$ Hz), 4.31 (2H, q, $J = 7.0$ Hz), 5.20–5.80 (2H, br), 7.47 (2H, t, $J = 7.4$ Hz), 7.55–7.64 (1H, m), 8.02 (2H, d, $J = 8.5$ Hz), 8.67 (1H, s). Compound **8**: mp 190–191 °C; ^{1}H NMR (300 MHz; TMS/$CDCl_3$) δ 1.39 (3H, t, $J = 7.1$ Hz), 1.75 (3H, d, $J = 7.1$ Hz), 2.62 (3H, s), 4.40 (2H, q, $J = 7.0$ Hz), 6.57 (1H, q, $J = 7.0$ Hz), 7.26–7.65 (6H, m), 7.70 (2H, d, $J = 8.7$ Hz), 8.04 (2H, d, $J = 8.8$ Hz), 8.77 (1H, s).

9. The ^{1}H NMR spectrum data of compounds **10** and **11** were as follows. Compound **10**: ^{1}H NMR (300 MHz; TMS/$CDCl_3$) δ 1.39 (3H, t, $J = 7.2$ Hz), 2.91 (3H, s), 4.37 (2H, q, $J = 7.2$ Hz), 5.51 (2H, s), 7.00 (2H, t, $J = 7.9$ Hz), 7.31–7.47 (4H, m), 7.68 (2H, d, $J = 7.6$ Hz), 8.37 (1H, s). Compound **11**: ^{1}H NMR (300 MHz; TMS/$CDCl_3$) δ 1.40 (3H, t, $J = 7.2$ Hz), 2.64 (3H, s), 4.38 (2H, q, $J = 7.1$ Hz), 5.34 (2H, s), 6.64 (2H, t, $J = 8.1$ Hz), 7.10–7.17 (3H, m), 7.36–7.47 (3H, m), 8.77 (1H, s).

10. Crystal data for compound **10**: $C_{23}H_{19}F_2N_3O_3$, $M_r = 423.42$, $\rho_{calcd} = 1.43$ gcm^{-3}, monoclinic, space group $P2_1/n$, $a = 15.812(3)$, $b = 14.321(4)$, $c = 17.544(2)$ Å, $\beta = 98.70(1)$, $V = 3926(1)$ Å^3, $Z = 8$; $R1 = 0.056$, $wR2$ (F^2; all data) $= 0.175$, $S = 1.05$ for 5838 unique data and 562 parameters, final difference synthesis max $= 0.55$, min $= -0.33$ eÅ^{-3}. Intensity measurement: Rigaku AFC5R diffractometer, Cu-K_α radiation, graphite monochromator, ω-2θ scan, $2\theta_{max} = 120°$, $T = 293$ K. Moderate decay of intensities was observed and corrected. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC 181750). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44–1223–336033; e-mail: deposit@ccdc.cam.ac.uk).

11. The physicochemical data of compound **15b** was as follows: mp 94–96 °C (free amine); ^{1}H NMR (300 MHz; TMS/$CDCl_3$) δ 1.36 (6H, d, $J = 6.3$ Hz), 2.17 (3H, s), 3.66 (2H, s), 3.83 (3H, s), 4.33 (2H, s), 5.19–5.27 (1H, m), 5.50 (2H, s), 6.99 (2H, t, $J = 8.1$ Hz), 7.02–7.26 (5H, m), 7.34–7.43 (1H, m), 7.58 (2H, d, $J = 8.7$ Hz), 8.03 (2H, d, $J = 8.7$ Hz), 8.37 (1H, s). IR (KBr) 1740, 1597, 1417, 1218, 1036 cm^{-1}. FAB-MS 645 (M + H). Anal. calcd for $C_{34}H_{34}N_6O_5F_2$: C, 63.34; H, 5.32; N, 13.04. Found: C, 63.64; H, 5.26; N, 12.86.

12. Receptor binding assays were carried out as described previously (see ref 4). Briefly, human LHRH receptor cDNA was cloned from a pituitary cDNA library and CHO cells stably expressing high levels of the recombinant human LHRH receptor were isolated. [^{125}I][Tyr^5]leuprorelin (0.12–0.15 nM) and the membrane fractions of the CHO cells (0.2 mg/mL) were incubated at 25 °C for 60 min in 0.2 mL assay buffer A [25 mM Tris, 1 mM EDTA, 0.1% bovine serum albumin (BSA), 0.03% NaN_3, 0.25 mM phenylmethanesulfonyl fluoride, 1 μg/mL pepstatin A, 20 μg/mL leupeptin and 100 μg/mL phosphoramidon, pH 7.5] containing various concentrations of the test compounds. The reaction was terminated by adding 2 mL ice-cold assay buffer A, and the bound and free ligands were immediately separated by filtration through a poly(ethylenimine)-coated glass microfiber filter (Whatman, GF/F). The filter was washed twice with 2 mL assay buffer A, and radioactivity was measured using a X-ray counter. Specific binding was determined by subtracting the nonspecific binding, which was measured in the presence of 1 μM unlabeled leuprorelin, from the total binding. The concentration of each test compound that produced 50% inhibition of the specific binding (IC_{50} value) was derived by fitting the data into a pseudo-Hill equation: $\log[\%SPB/(100-\%SPB)] = n[\log(C) - \log(IC_{50})]$, where %SPB is the specific binding expressed as a percentage of the maximum specific binding; n is the pseudo-Hill constant; and C is the concentration of the test compound.

13. Inhibition of LHRH-stimulated arachidonic acid release from CHO cells expressing human LHRH receptors was measured to evaluate the functional LHRH antagonism of the test compounds according to the previously reported protocol; see: Masuda, Y.; Sugo, T.; Kikuchi, T.; Kawata, A.; Satoh, M.; Fujisawa, Y.; Itoh, Y.; Wakimasu, M.; Ohtaki, T. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 675. The human LHRH receptor-expressing CHO cells were seeded into 24-well plates at a density of 4×10^4 cells/well and cultured for 1 day. The cells were then incubated with [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (11 kBq/well, NEN Lifescience Products) for 1 day and washed with Dulbecco's modified Eagle's medium (DMEM) supplemented with 20 mM HEPES and 0.2% BSA. The cells were then pre-incubated with the compounds at 37 °C for 60 min and the reaction was started by addition of LHRH (1 nM). After incubation at 37 °C for 40 min, radioactivity in the medium was measured with a liquid scintillation counter. The assays were repeated twice. IC_{50} value is the antagonist concentration required to inhibit the LHRH-stimulated arachidonic acid release from CHO cells by 50%.